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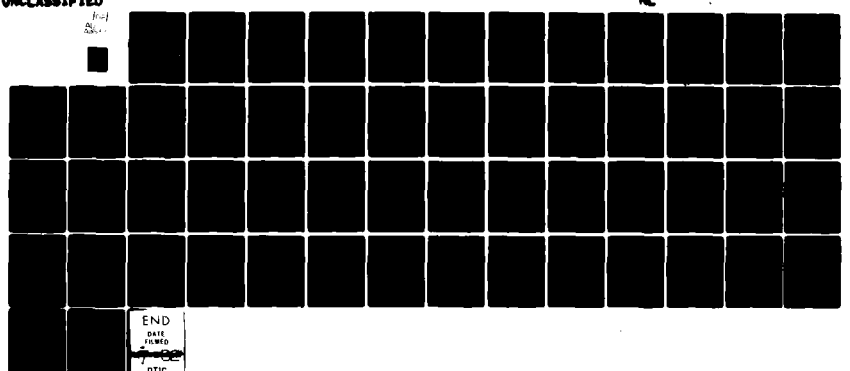

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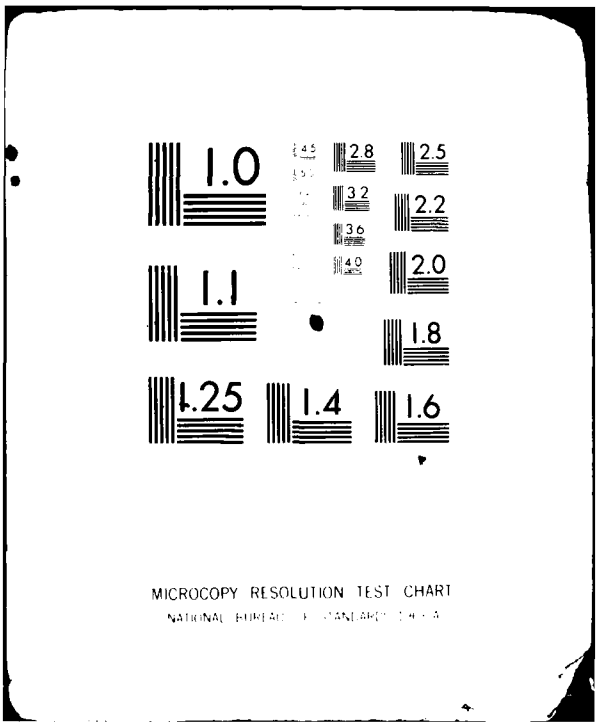
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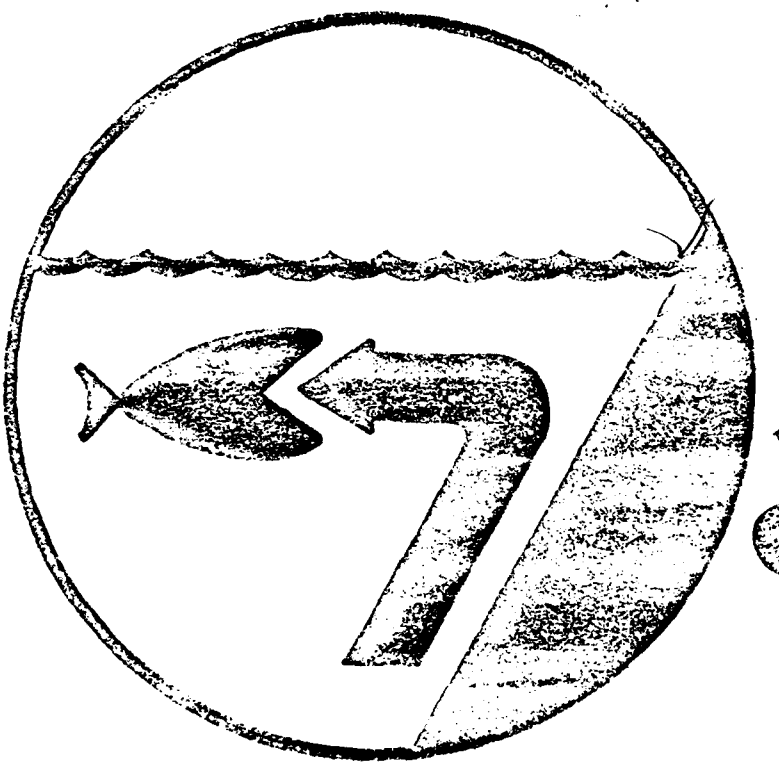
INTERNATIONAL DECADE OF OCEAN EXPLORATION

COASTAL UPWELLING ECOSYSTEMS ANALYSIS

Technical Report 48

AUTOMATED CHEMICAL ANALYSIS FOR MEASURING RESPIRATORY ELECTRON TRANSPORT ACTIVITY IN MARINE PLANKTON



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J. Abrahamson, F. Setchell, V. Jones, and T. Packard

January 1980

Automated Chemical Analysis for Measuring Respiratory Electron Transport Activity in Marine Plankton

By

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CUEA TECHNICAL REPORT NO. 48

Contribution No. 79034 from the Bigelow Laboratory

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OBJECTIVE

The objective of this research was to develop an automated system to monitor microplankton respiration via analysis of ETS activity. This system would facilitate the processing of the large number of seawater samples that are characteristically taken on ocean survey cruises. Furthermore, it would permit the construction of maps of plankton metabolism.

SUMMARY OF THE OPERATING SYSTEM

The purpose of the present automated ETS design is to standardize the timing and performance of all the manipulations and transfers in the chemical phase of the enzyme assay. Other than the thermostat, the machine has no feedback system. However, steps have been taken to minimize the effect of small variations in pumping rates, sample volumes, or turbidities on machine operation. The machine can be modified easily to accommodate improvements in the enzyme assay.

The device is designed for computer interface in a manner similar to the nutrient Autoanalyzer^{R} array. It has a potentiometric output, 0-1 V linear with 0-1 Absorbance from the spectrophotometer. The starting switch can flag the Data Acquisition System (DAS), in which case the DAS clock takes readings every 15 seconds from one minute to four minutes thirty seconds. The enzyme reaction in the spectrophotometer cell is zero order and linear during the time of observation.

A linear regression on the slope of the recorder trace yields the rate of reaction. The device does not free the analyst of all hand manipulations. The cell free homogenate must be prepared by hand and injected into the mixing chamber. Fifteen seconds after starting, a pair of syringe pumps (driven by another pair reversed and subject to pressure and vacuum) adds the two reagent mixtures (Fig. 1). The reagents are preheated, mixed for 15 seconds in the mixing chamber with the homogenate, and then drawn into the temperature controlled flow cell where the mixture is held for recording. Later the excess is removed from the mixing chamber and the mixing chamber and flow cell are rinsed with distilled water. All of the systems return to their base state quickly and are ready again for immediate use without lag. One complete cycle takes five minutes.

DESCRIPTION OF THE INSTRUMENT

Syringe pumps

To dispense measured quantities of reagent, a simple pump (Fig. 2) was built from 2 cc glass syringes and a self-refilling valve. Each pump was made by coupling the plungers of two syringes to each other by a bar-bell shaped connecting rod. Each bead on the end of the connecting rod was coupled tightly to the plunger by a piece of latex tubing to make a pair of semi-universal joints that were strong enough to pull the syringe during the refilling process. The barrels of the two syringes were mounted so that the axis of all the parts fell on a vertical line and the amount of travel between the

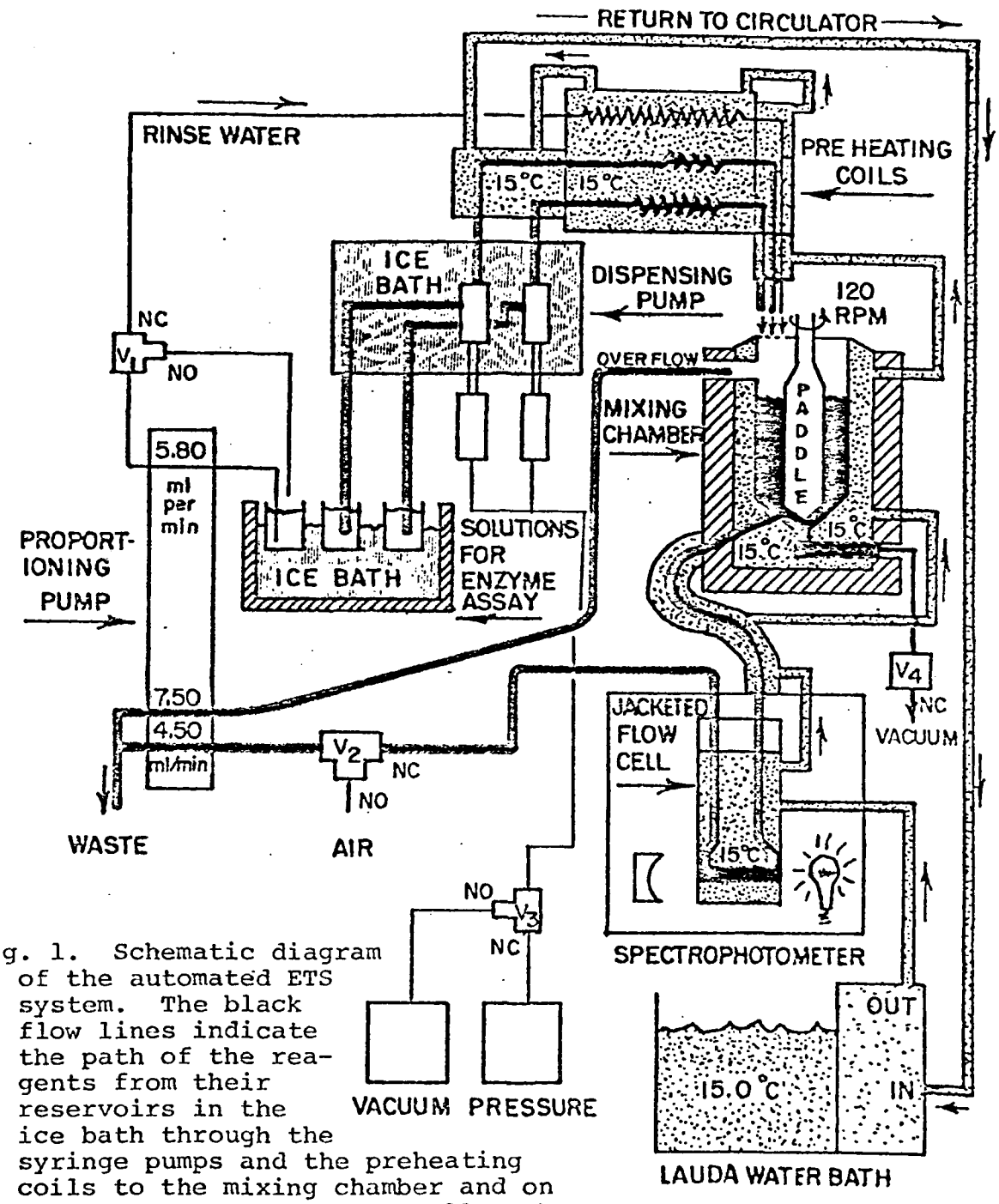


Fig. 1. Schematic diagram of the automated ETS system. The black flow lines indicate the path of the reagents from their reservoirs in the ice bath through the syringe pumps and the preheating coils to the mixing chamber and on to the spectrophotometer cell. The stippled flow lines indicate the paths of the cooling water from the Lauda water bath through the spectrophotometer cell (jacket) around the mixing chamber and preheating coils and back to the Lauda water bath. The normal functioning of each of the four solenoid valves is indicated by NO (normally open): NC indicates the normally closed path. The proportioning pump was a Technicon Autoanalyzer pump and the spectrophotometer was a Bausch and Lomb Spectronic 88.

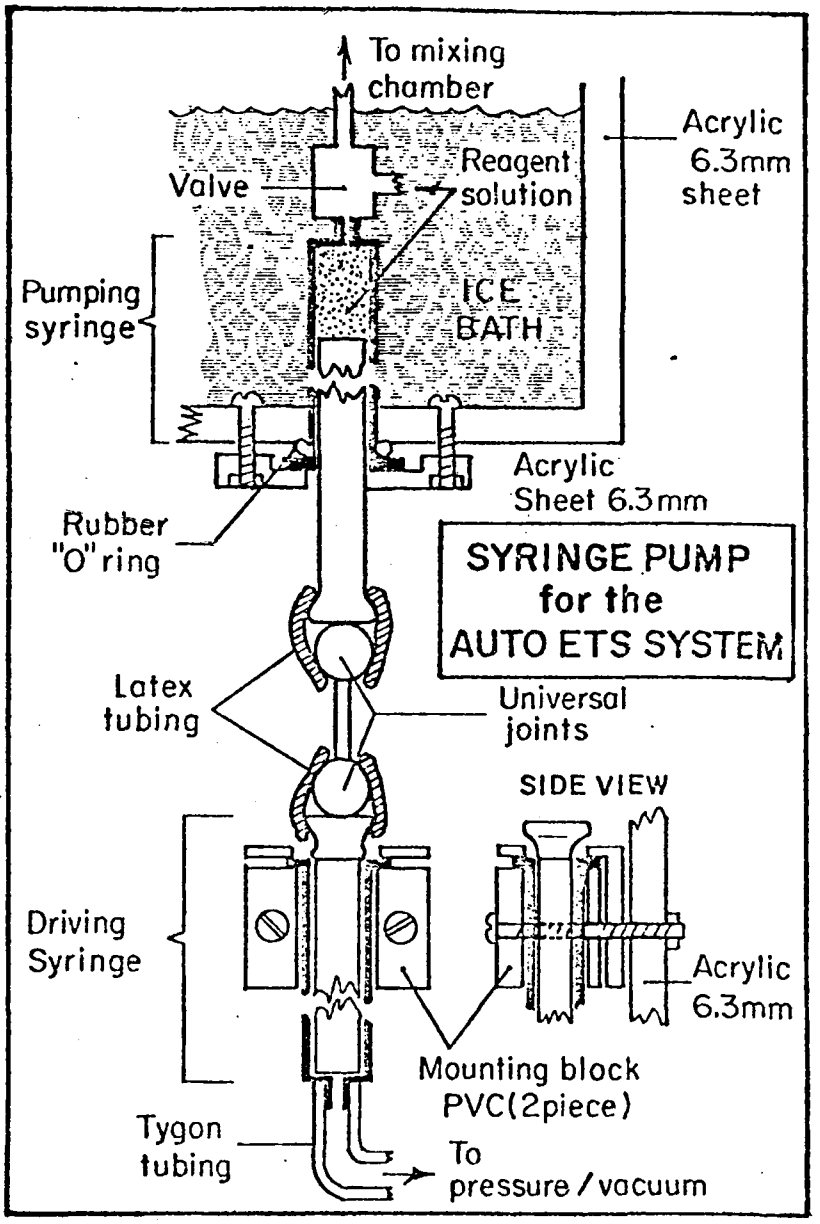


Fig. 2. Syringe pump (frontal view) for delivering precise reagent volumes to the mixing chamber. The driving syringe is connected via a solenoid valve (V_3 in Fig. 1) to a pressure-vacuum system. The upper part of the pumping syringe is maintained in an ice bath during use.

coupled plungers corresponded to the desired delivery volume. The self-refilling valve was mounted on the top syringe. The bottom syringe was connected to a valve (V_3) that connected either to a vacuum of 390 mm Hg or to a pressure of 528 mm Hg. To deliver the volume of reagent it was necessary only to switch to pressure for a long enough time to push the plungers to the end of the syringe barrel and then to switch to vacuum for refilling. Special care was taken to insure that the close fitting parts were clean and properly matched. Otherwise, the syringe pumps would not operate smoothly. To preserve reagents, the reagent reservoirs and pumps were packed in ice.

Pre-heating reagent delivery system

The delivery lines from the syringe pumps to the mixing chamber (Fig. 1) were warmed to the temperature of the enzyme reaction. Those lines were size 22 TW Teflon tubing. They travelled through flexible tubular insulation with the 3.2 mm ID Tygon circulator tubing. In addition, each line included a jacketed glass coil which held more than one measured volume of the reagent. The various connections were made by sleeving different sizes of tubing. The reagent delivery lines were terminated by 21 gauge hypodermic needles clamped in place over the mixing chamber so that their effluent streams were directed into the bottom of the chamber (Fig.1).

Mixing chamber

The mixing chamber served two functions. The first was to start each assay by holding all of the components together and mixing them (a small Teflon paddle turned at 120 rpm). The chamber was temperature controlled by copper tubing wrapped about it and insulated (Fig. 3). Water was passed

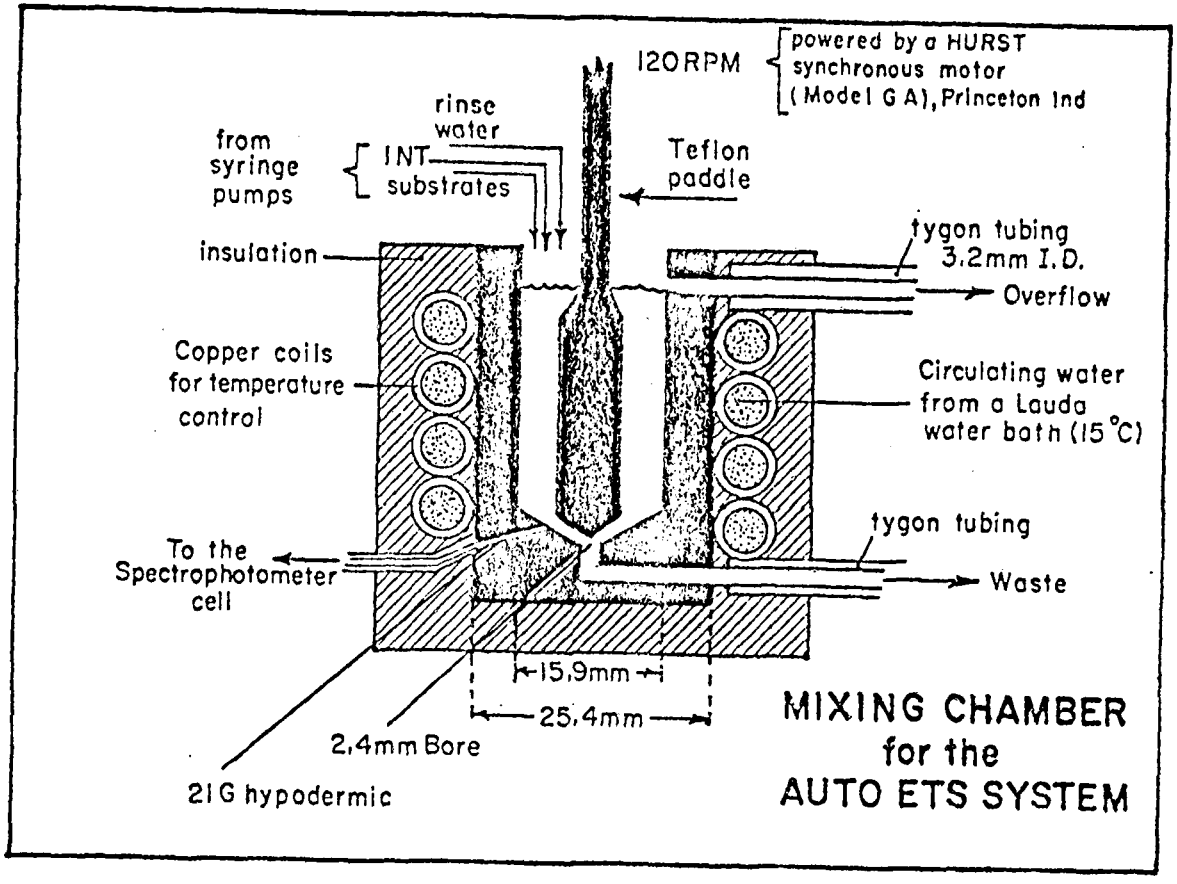


Fig. 3. This mixing chamber was used to blend the substrate solution, the INT solution, and the enzyme homogenate. After blending, the mixture was drawn into the spectrophotometer cell for kinetic analysis. The chamber was kept at 15°C by the Lauda water bath. The chamber and paddle were milled from a Teflon rod.

through that tubing at incubation temperature. The second function of the chamber was to insure the separation of individual assays from each other and from the deionized water used to wash the reaction vessels in between. This more than anything else governed the design of this part of the device.

The chamber was constructed from a 89 mm piece of 25.4 mm Teflon roundstock with a 15.9 mm diameter hole drilled 76.2 mm along its axis leaving the bottom pointed like the drill. At that point there was a 2.4 mm hole that met another of the same diameter that was drilled in from the side of the chamber. This hole was connected to an aspirator through a normally closed (NC) solenoid valve (V_4). When this valve was opened the chamber was quickly sucked dry from the bottom. The Teflon allowed little of the mixture to cling to it. A hole large enough for a 21 gauge hypodermic needle opened into the chamber halfway up the conical bottom (Fig. 3). The size and position of this hole were important. It had to be above the drain so that no traces of the old reaction mixture could be trapped in it but near enough to the bottom so that most of the reaction mixture could be drawn into the flow cell. The opening had to be as small as possible to minimize the contact between the solution entering the chamber and the rinse water that remained (flow-cell line). The open top of the mixing chamber did not allow the aspirator to evacuate the flow-cell line and it remained filled with distilled water. Near the top of the mixing chamber was a 3.2 mm overflow that drained any excess reaction mixture. The line between the mixing chamber and the flow cell was

made of 22TW Teflon.

The flow-cell was a glass spectrophotometer cuvette (Fig. 4) called a special Smith-Kline cell by its manufacturer, Hellma Cells. It fitted into a common rectangular cell holder and had an unmasked lightpath 10mm long and 3 mm in diameter. From either end of the lightpath a small bore tube ascended creating the flow path. There was also a water jacket around the sides and bottom of the lightpath that ended in two nipples near the top of the cell. The effluent side of the flow cell was connected to the normally closed (NC) side of a valve (V_2) that was normally open (NO) to the air so that the pump tubing on the Technicon Autoanalyser pump I that was connected to the third opening on the valve will draw air and the tubing will not become evacuated between assay. This design prevented irregular flow and provided a beneficial time lag before the solution began to move at 4.50 ml/min. When the valve switched from air to the incubation cell, the pump pulled liquid from the mixing chamber through the flow-cell to the waste container.

Temperature control

To maintain incubation temperature throughout the various parts of the machine, water was circulated through an insulated loop by a Brinkmann-Lauda K/2-R constant temperature circulator. The water flowed first around the incubation cell, then around the mixing chamber, around the glass coils, and finally along the transmission lines. The circulator was set at the maximum pumping rate but the flow rate was limited by the size of the incubation cell jacket.

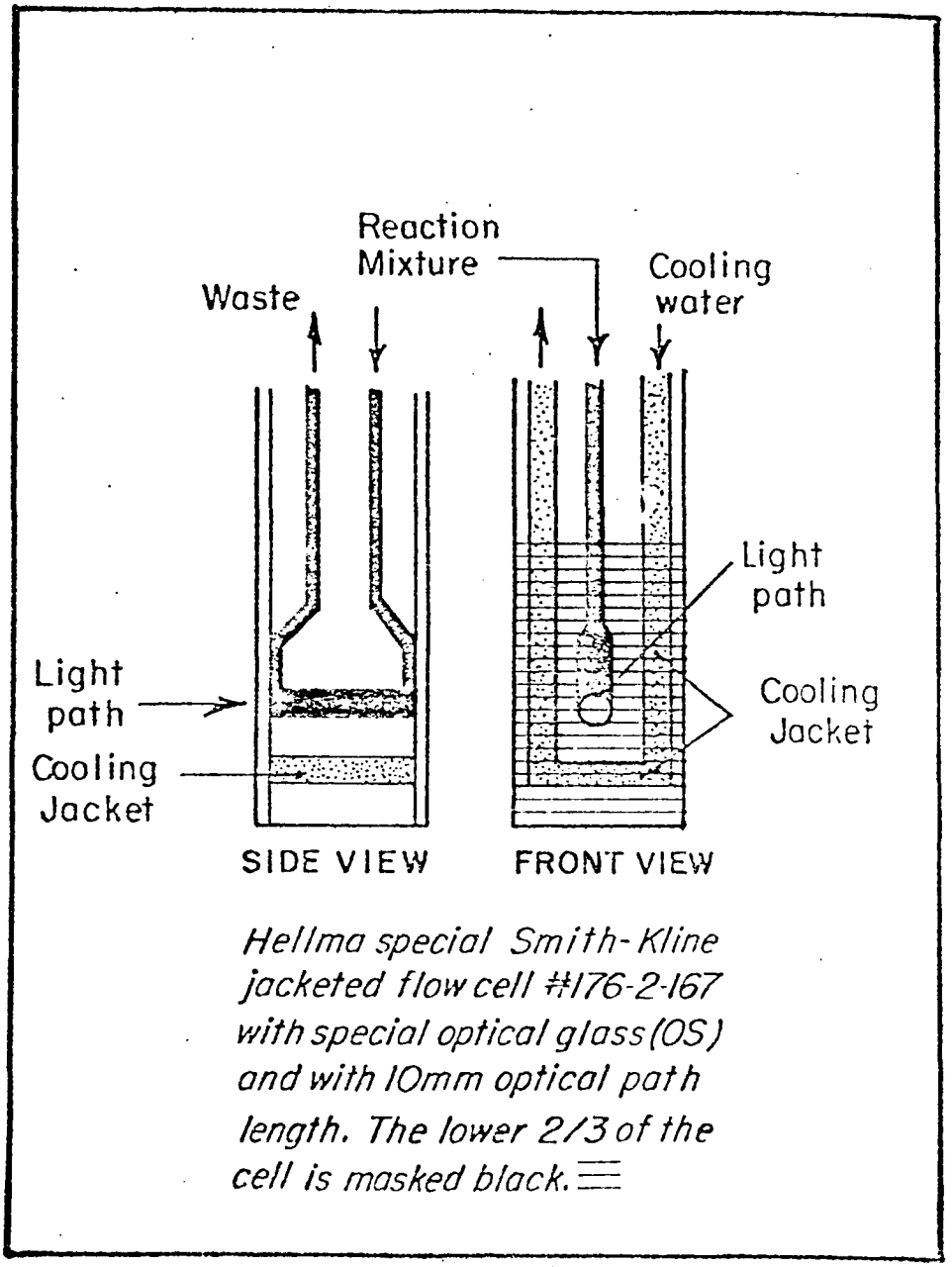


Fig. 4. This jacketed flow cell was installed in the spectrophotometer.

Rinsing

After each assay it was necessary to clean the mixing chamber, incubation cell, and the connecting tube so that these parts did not become stained and so that the conditions preceeding each assay wereidentical. For that purpose, a reservoir of deionized water was kept in an ice bath with the reagent solutions and was pumped to the mixing chamber in the similar manner as were the reagents, except that the Technicon pump was used. A valve (V_1) was provided so that the pump's flow was directed back to the reservoir when washing was not needed and the pathway to the mixing chamber was closed (NC). To wash the system the mixing chamber was first evacuated by opening valve V_4 and then rinsed with deionized water by opening valve V_1. The proportioning pump maintained the influent flow of the rinse water at 5.80 ml/min. and the effluent flow to 4.50 ml/min. The imbalance in the flow rates was necessary to maintain sufficient liquid in the mixing chamber to prevent air from entering the flow cell. The introduction of air bubbles to the flow cell caused large undesirable excursions in the absorbance. Afterwards both valves, V_1 and V_2, were closed and the mixing chamber was evacuated and the system was ready for the next measurement.

The flow of reagents throughout the machine was controlled by four solenoid valves and by the pumping rates. The valves were controlled by a stepping switch that closed each of twenty connections sequentially for equal time intervals. This switching circuit (Fig. 5) was set for 15 second

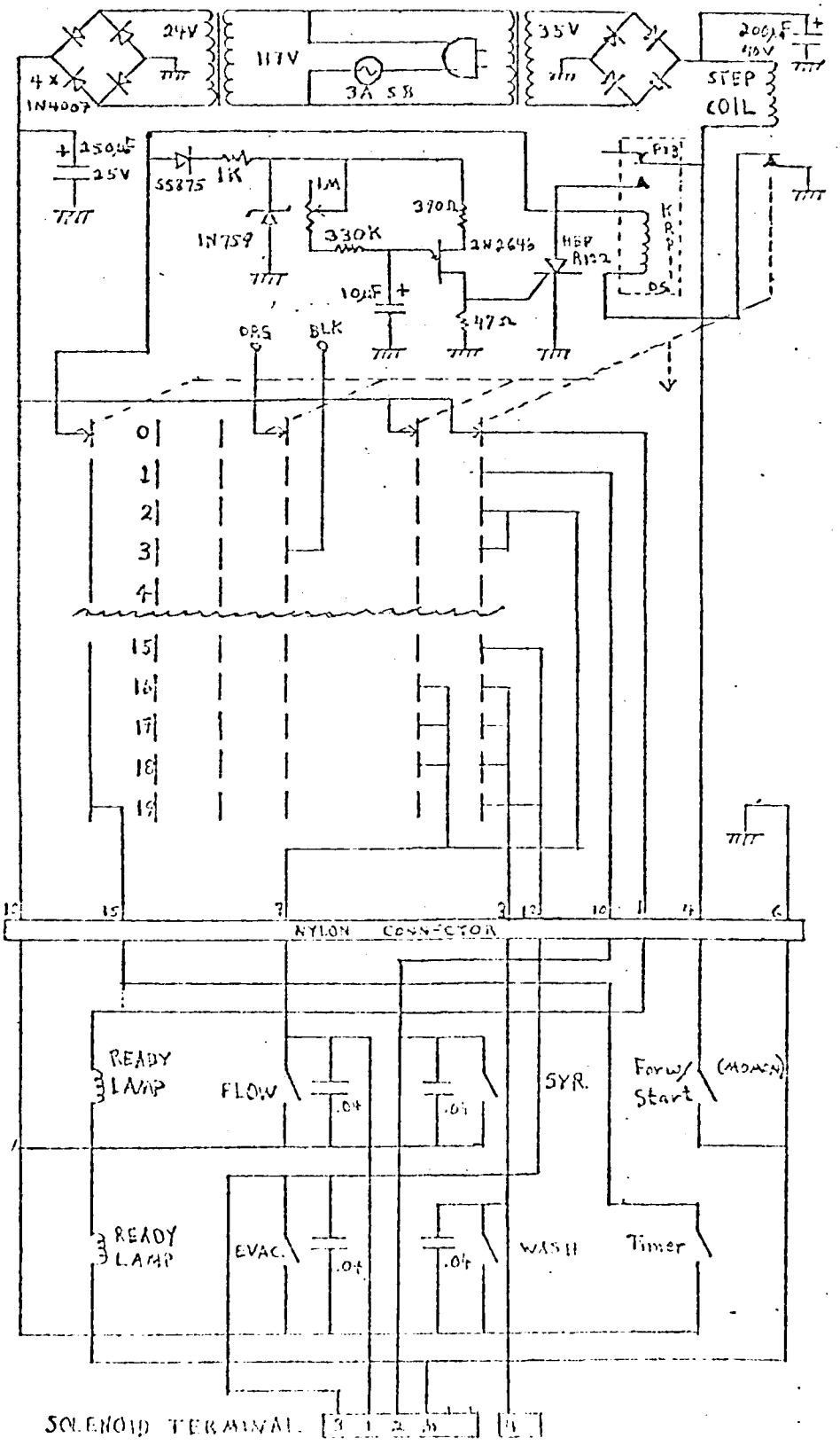


Fig. 5. Wiring diagram for the reagent control mechanism in the auto EIS system.

were adjusted so that an integral number of intervals would activate the pumps and valves for the required time. At the end of twenty steps (5 minutes) the switch returned to the initial position and remained in that position until it was reactivated. Modifying the enzyme assay could be accomplished by rewiring the switch. The switch had alternate sets of contacts that enabled valves to be independent of each other even though they shared some switch positions.

The spectrophotometer was a Bausch and Lomb Spectronic 88 with a single cell compartment that had been slightly modified by insulation and by cutting a pivot pin so the door could be opened without moving the cell. The circulator and reaction flow lines entered the compartment through a hole in the top of the instrument. The external signal output used was 0-1 V linear with 0-1 Absorbance units. The signal was displayed on a meter on the front of the spectrophotometer as well as on a Hewlett Packard 7100A chart recorder with a 0-1 V range.

ETS ACTIVITY MEASUREMENT

The rate of INT reduction in the flow cell was measured by the absorbtion of the reaction mixture at 490 nm as described in Kenner and Ahmed (1975a). The reaction was monitored for 3 min. after the reaction mixture was introduced into the flow cell. The slope of the trace on the chart recorder was the reaction rate (or activity) in units of absorbance per minute. A blank was made up and measured in the same manner as an assay except that homogenization buffer was substituted

for homogenate in the reaction mixture. The average of several blanks was subtracted from the assay to obtain the rate of the enzymatically catalysed INT reduction.

CALCULATIONS OF ACTIVITY

To calculate the ETS activity it was necessary to account for the volume of seawater sampled, the volume of the homogenate, the volume of the reaction mixture, and the absorbance of the INT equivalent of one microliter of O_2 (Kenner and Ahmed, 1975). The following equation was used for calculating ETS activity measured by the automated method. The ETS activity is in units of $\mu l\ O_2 h^{-1} l^{-1}$.

$$ETS = \frac{60 \times S \times H \times (A-B)}{f \times V \times 1.42}$$

H is the volume of the uncentrifuged homogenate in milliliters less the volume of the inert glass filter particles (0.1 ml). A is the change in absorbance of the 10 mm light path per minute (slope of the line on the recorder), B is the slope of the control assay, V is the seawater volume (liters) that was filtered, 60 is the number of minutes per hour, S is the volume of the reaction mixture (2.5 ml), f is the volume of homogenate injected into the mixing chamber (0.5 ml), and 1.42 is the absorbance equivalent of one microliter of O_2 (Kenner and Ahmed, 1975).

Temperature correction

The *in situ* temperatures of the seawater samples vary with depth and surface location. To report the ETS activity at *in situ* temperature it was necessary to use a calculated correction because it was unfeasible to readjust the incubation temperature for every sample. To this end the Arrhenius equation was employed (Packard, King, and Devol, 1975). The equation employed was:

$$ETS_{in\ situ} = ETS_{incubation} \exp \frac{15800}{R}\left[\frac{1}{T_{inc}} - \frac{1}{T_{in\ situ}}\right]$$

where R is the gas constant (1.987 cal $mole^{-1}deg^{-1}$) and 15800 is the activation energy in cal $mole^{-1}$ (Packard, King, and Devol, 1975). Both the incubation (T_{inc}) and *in situ* temperatures ($T_{in\ situ}$) were expressed as absolute temperatures.

OPERATING INSTRUCTIONS

Introduction

The automated ETS assay system is a laboratory built, kinetic enzyme-analysis system dedicated to measurements of the respiratory electron transport system in cell free homogenates. The system resembles other kinetic enzyme-analysis systems marketed by Bausch and Lomb, Beckman, etc. By making certain adjustments it may be possible to assay other enzymes. However, the automated ETS assay system was designed and built with the single dedicated purpose in mind of making ETS measurements.

The time and effort involved in setting up and running the system, as well as the reagents necessary for priming the system, make it economical to use only when approximately 10 or more samples are to be run in one day.

Reagents

All reagents are prepared as in Kenner and Ahmed (1975a) for phytoplankton assays or Owens and King (1975) for zooplankton assays. Additional grinding buffer is needed for dilution for zooplankton samples. Needed are:

1. Homogenizing buffer (HB)
   (NaCN, if used, to be added after defrosting)
2. Substrate Buffer (SB)
   (with Na Succinate added but no NADH or NADPH which are added after defrosting)
3. INT Solution
4. NaCN 0.1 M solution
5. Preweighed substrates (NADH, NADPH)

NOTE: Bulk preparation of solutions is recommended. Individual screw capped plastic bottles are used to store prepared solutions frozen. They should contain sufficient material for one day's operation or an integral submultiple.

Reagents needs per sample including 1 blank for each 10 samples, are as follows:

Homogenizing Buffer	3.05 ml
Substrate Buffer	1.65 ml
INT Solution	0.55 ml

In addition to the above requirements volume for priming the pumping system, initial blanks, and homogenate dilution (in the case of zooplankton samples) are needed.

Thus, for 25 phytoplankton samples the following are suggested:

Homogenizing Buffer (HB)	90 ml
Substrate Buffer (SB)	55 ml
INT Solution	25 ml

Frozen solutions should be thawed in water and additional reagents added as needed. Once defrosted and additions made they should be kept on ice.

Set Up

The circulating water bath should be set to the desired temperature (often 15°C) and switched on 45 minutes to 1 hour before beginning assays to permit proper attainment of incubation temperature.

The Spectronic-88 spectrophotometer should be turned on approximately 30 minutes before assays are begun. It is an extremely stable instrument and should seldom require any adjustment if it is allowed to warm up before beginning the assays.

Crushed ice for the reagent storage reservoir should be placed in the bucket on which the syringe-pumps are mounted. The prepared reagents and rinse water reservoir should then be placed in the bucket and the appropriate lines inserted into them. (CAUTION - be sure to place both rinse water lines in the rinse reservoir.) An additional bucket of ice is used to store the HB and homogenates in the work area.

Just before beginning assays, the proportioning pump, vacuum pump, and other electrical equipment are turned on and the N_2 pressure system is set to 20 psig. The syringe pumps

and reagent lines are primed by first turning the mixing chamber evacuation line on (switch labeled "EVAC"), then either manually moving the syringes up and down 5-6 times or using the "SYRINGE" switch. After so doing, the mixing chamber and flow cell are rinsed (throw "WASH and "FLOW" switches) for 30 seconds to 1 minute. Shut off "FLOW" and evacuate mixing chamber when finished.

The chart recorder speed is set for one small chart division per minute (on the Hewlett-Packard recorder, 6 in $\cdot$ h^{-1}). The spectrophotometer is then zeroed and the zero checked on the chart and adjusted as necessary. The spectrophotometer should be adjusted so that the meter reads 0.1A.

NOTE: The purpose of setting the meter to 0.1A is to allow below-the-baseline excursions on the chart recorder and meter. Since reaction rates are based upon the slope of the recorder trace, not absolute absorbance measurements, this setting has no effect upon the results - it merely protects the recorder and meter from possible damage due to being "pinned" off scale.

Blanks and Samples

A calibrated pipette (e.g. Selectapette or Eppendorf) is used to dispense 0.5 ml homogenizing buffer into the mixing chamber. The "START" switch is then depressed and the system will automatically begin the assay cycle. Three (3) or four (4) initials blanks are generally run, until the slopes are visually identical. Regular samples can now be run.

Phytoplankton samples are filtered, ground and centrifuged as in Kenner and Ahmed (1975a). Zooplankton samples are processed and diluted as in Owens and King (1975). A subsample (0.5 ml) of the cleared homogenate is pipetted into the mixing chamber and the cycle started by depressing the "START" switch.

A blank is run for every 10 samples and at the end of the run.

Shut Down

At the conclusion of the day's run the system is thoroughly flushed with distilled water, as in the priming operation, paying particular attention to the syringe pumps. All parts of the apparatus are shut off (including N_2 tank) and the roller head on the proportioning pump is released.

TROUBLESHOOTING

The automated ETS system is, by and large, an extremely reliable machine which has suffered few significant failures in over 5 years of operation, including several months at sea. However, it has a few idiosyncrasies which, if understood, will lead to excellent data production. Specifically not covered in this section are problems associated with the Spectronic-88 spectrophotometer for which an excellent service manual exists.

Unstable Baseline

Occasionally, a bubble or bubbles will become lodged in the light path of the flow cell. This may be observed as a baseline which does not return to zero at the conclusion of a cycle or unusual noise in the recorder trace, particularly when liquid is flowing.

The cure is simply to evacuate the mixing chamber by turning on the "EVAC" switch briefly and turning on the "FLOW" switch with the chamber empty for about 5 seconds. Then the "WASH" switch is turned on and distilled water is pumped through until the baseline is back to normal. Usually a single treatment will resolve the difficulty though repetition may be necessary.

Rising Baseline

On warm, humid days when the incubation temperature is below the dew point, one may observe a rising baseline as a result of condensation of water on the flow cell. To

correct this problem, one may either raise the incubation temperature (usually undesirable) or blow dry gas (N_2 from the pressure system) through the sample chamber at a rate just sufficient to prevent condensation. Dessicants have not been used successfully because of the many air leaks into the chamber though it is conceivable that one could seal the chamber better and maintain dryness; the absorptive rate of most dessicants is not rapid enough to quickly dry out even a sealed chamber.

Reagent Dispenser Problems

Perhaps the most trouble prone parts of the AETS system are the syringe pumps. They should be observed during operation at the beginning of the cycle and to see if they properly refill. They must be kept scrupulously clean and properly aligned to prevent sticking.

Proportioning Pump Problems

In order to maximize pump tube life, the operator must be sure to release the roller head at the end of a day 's run. The pump tubes should be changed when they are visibly distorted in shape.

COMPARISONS WITH THE METHOD OF KENNER AND AHMED

Comparison of the automated analyzer's machine's results with those of the parent technique (Kenner and Ahmed, 1975a) provided information on how well the analyzer duplicated it. The following is the procedure used by Kenner and Ahmed (1975a). The solutions are the same except as noted. Three ml of substrate solution and the substrate solution's buffer are placed in glass tubes on ice. One ml of homogenate is added to each and the reaction is started by adding 1 ml of INT solution. The tubes are incubated in a water bath covered by serum stoppers. After 20 minutes the reactions are stopped by adding 1 ml of quench solution to each tube. The quench solution consists of a 1:1 (vol:vol) solution of concentrated formalin and a 1 M solution of sodium formate at pH 3.5.

The time and the volumes in the tubes (6 ml) are noted. A blank is performed in the same manner except that buffer is substituted for homogenate. A change in absorbance (ΔA) is calculated by subtracting the absorbance at 490 nm of the tube without substrate from that of the tube with substrate. Dividing the result by time and other relevant quantities, ETS activity is calculated by inserting the ΔA in the following equation:

$$\text{ETS } (\mu l\ O_2\ l^{-1}h^{-1}) = \left(\frac{60 \times S \times H \times \Delta A}{T \times V \times 1.42} - \frac{60 \times S' \times H' \times \Delta A'}{T' \times V' \times 1.42} \right)$$

where H is the homogenate volume, T is the reaction time, V is the seawater filtered, S is the final volume in the assay tubes, and 60 is the number of minutes in an hour. All quantities designated by (') are those associated with the blank. The time (T) is in minutes, V is in liters, and all the other

volumes are in milliliters. Essentially this measurement is the average change in absorbance over about twenty minutes in the presence of the homogenate less the average change over twenty minutes without it. Temperature corrections, if necessary, were made as described above.

Depth profiles of ETS were used on JOINT I using water samples taken at six light depths from 100% to 1% at about 0900 h. Two liters were taken from each depth and stored briefly in four liter polyethylene bottles in deck seawater incubators. 100%, 50% and 30% were exposed to sunlight and 15%, 5% and 1% were kept in the dark. Homogenates were made as described above starting with the 100% sample and finishing with the 1% sample 1 to 1.5 hours later. On four occasions comparisons of the automated method and the manual method were made. Two of these were subject to serious non-random errors and were not reported. The other two are presented here (Fig. 6). Three preliminary experiments were performed during Leg 0 of JOINT I but they do not exhibit any correlation between the two methods. This may have been due to differences in the analysts (E. Wold performed the Kenner and Ahmed, 1975a, measurements and J. Abrahamson ran the automated ETS system) or to the efficiency of the machine. The Leg I profiles obtained by the two methods (Fig. 6) were similar.

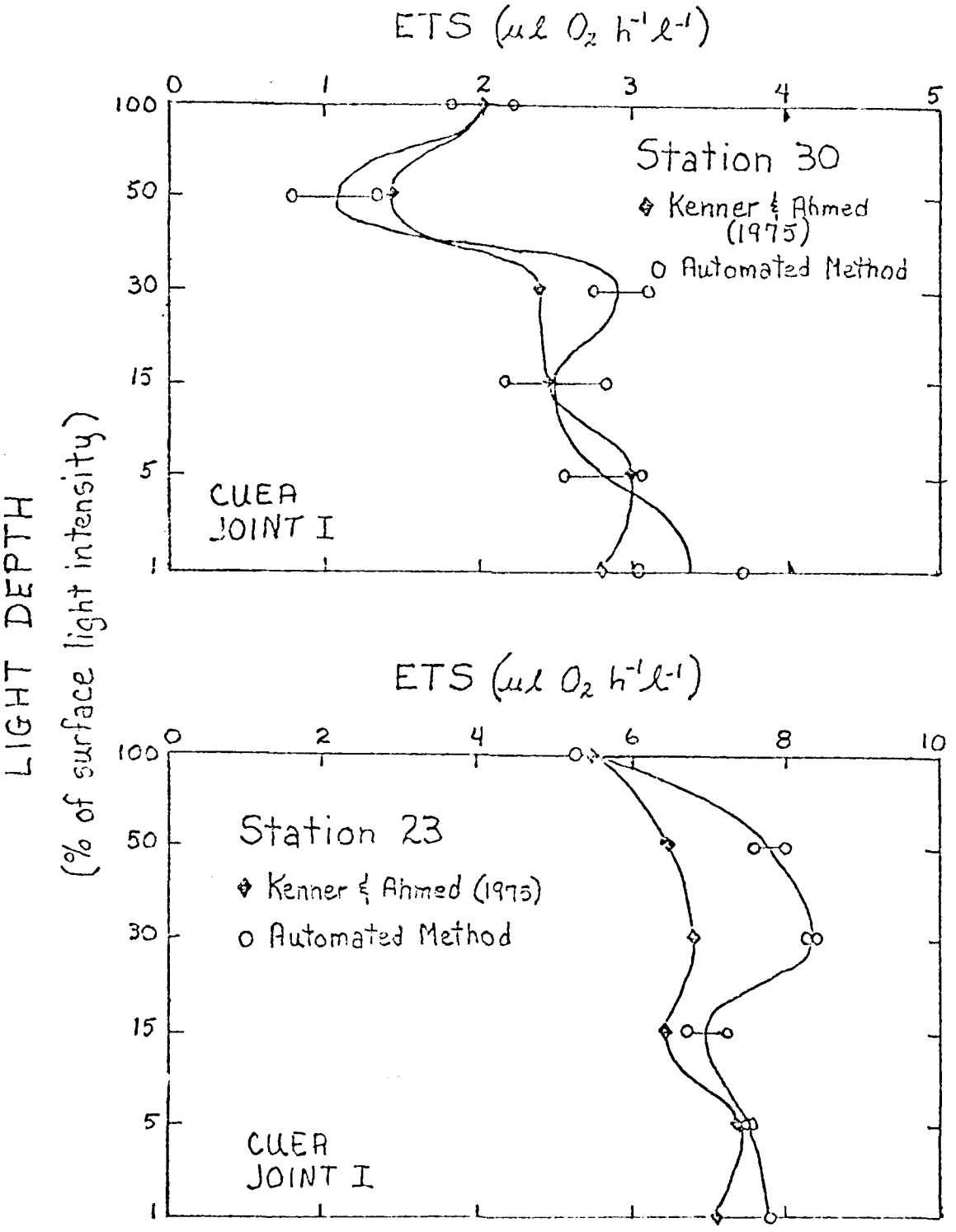
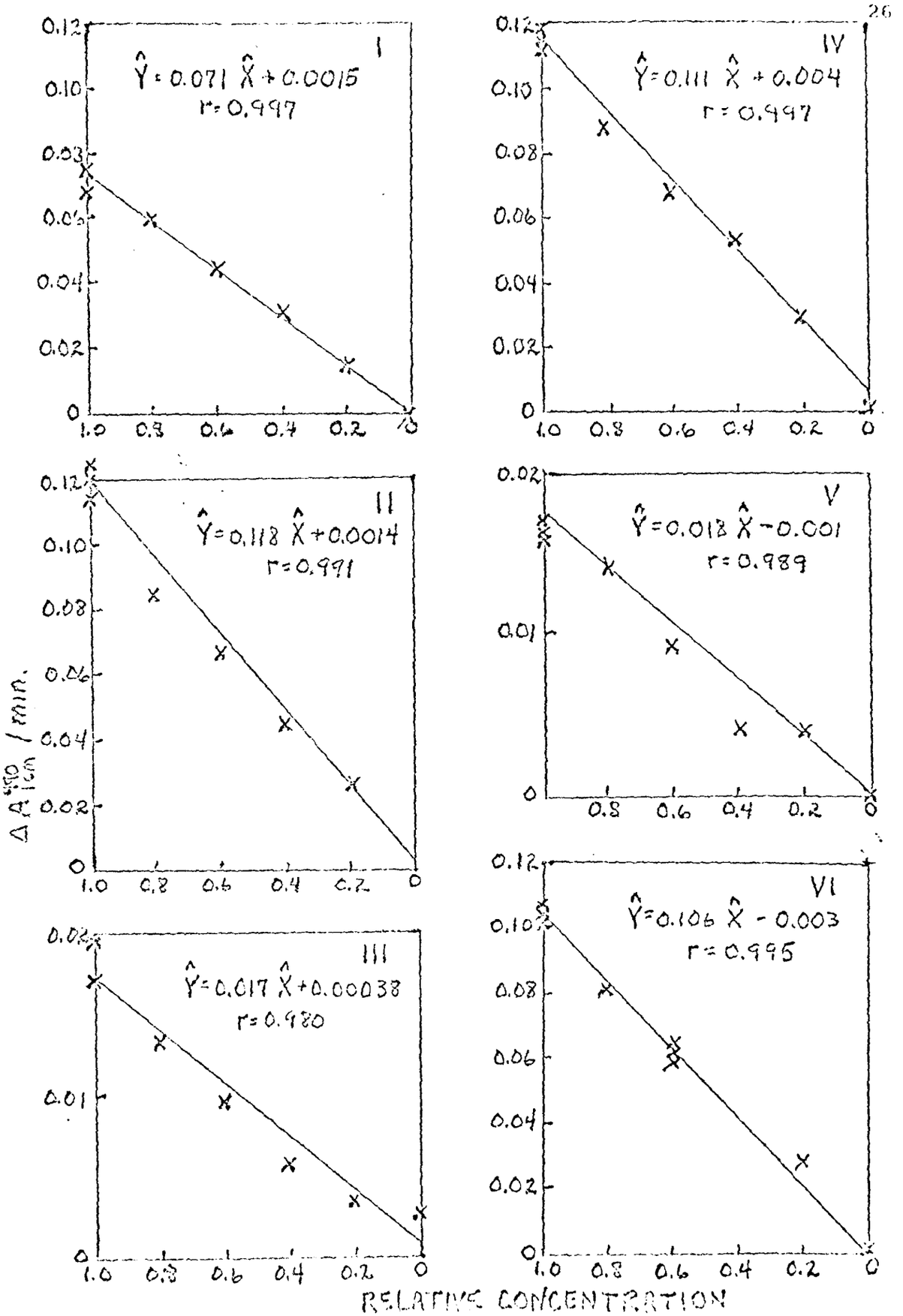


Fig. 6. Comparison of ETS depth profiles measured by the manual and by the automated methods.

DETERMINATION OF LINEARITY AND PRECISION

Both precision and enzyme-dependent linearity can be determined simultaneously from assays on serial dilutions of the same homogenate. This was done on chemostat cultures of *Skeletonema costatum*. Homogenates were prepared from a dense culture (~$2 \cdot 10^8$ cells/l) containing a normal mixture of sexually and asexually reproducing forms (P. Harrison, personal communication). The culture was maintained at 17-18°C as described by Harrison (1973). Six samples from the chemostat outflow were collected randomly and homogenized as described by Packard (1971). Subsamples ranging from 0.1 to 0.5 ml were diluted to 0.5 ml with homogenizing buffer, when necessary, and assayed. ETS activity was reported as change of absorbance at 490 nm per min ($\Delta A^{1\,cm}_{490}$)/min. The absorbance changes were corrected for changes in the blank. All six experiments (Fig. 7) show linearity and the high correlation coefficients indicated high precision. A numerical value for precision can be calculated by normalizing each ΔA value by the original homogenate volume used.

Fig. 7. Precision and linearity experiments using the auto-ETS method. Serial dilutions of cell-free homogenates were prepared from Skeletonema costatum cultures. ETS activity (ΔA_{1cm}^{490}/min) was then measured in each dilution.


I
$\hat{Y} = 0.071\ \hat{X} + 0.0015$
r = 0.997
IV
$\hat{Y} = 0.111\ \hat{X} + 0.004$
r = 0.997
II
$\hat{Y} = 0.118\ \hat{X} + 0.0014$
r = 0.991
V
$\hat{Y} = 0.018\ \hat{X} - 0.001$
r = 0.989
III
$\hat{Y} = 0.017\ \hat{X} + 0.00038$
r = 0.980
VI
$\hat{Y} = 0.106\ \hat{X} - 0.003$
r = 0.995
ΔA^{450}_{1cm} / min.
RELATIVE CONCENTRATION

UNDERWAY SURFACE ETS MAPS

Contour maps (Figs. 8-15) of ETS activity at the 3 meter level off the coast of Spanish Sahara were prepared during JOINT I using the contouring package of the IRIS computer system onboard the *R/V ATLANTIS II* (IRIS Users Manual). Table 1 gives the coordinates of these maps. The data for these ETS surface maps were collected using the automated system and homogenate preparation methods described above. Two liter seawater samples were collected from the ship's pumped aquarium water system at a rate of four to eight samples each hour while the ship steamed along its nighttime mapping track at eight to ten knots. Such maps usually lasted from 2000 to 0700. Concurrently the IRIS system was also taking data on the ship's position, certain nutrient concentrations, temperature, salinity, and *in vivo* fluorescence (Lorenzen, 1966). A summary of the type of data collected is shown in Table 2. Interpolated ETS and chlorophyll-a data (*in vivo* fluorescence) were compared to each other and correlation coefficients of those regressions were obtained (next section).

Figs. 8 - 15. Maps of microplankton ETS activity.

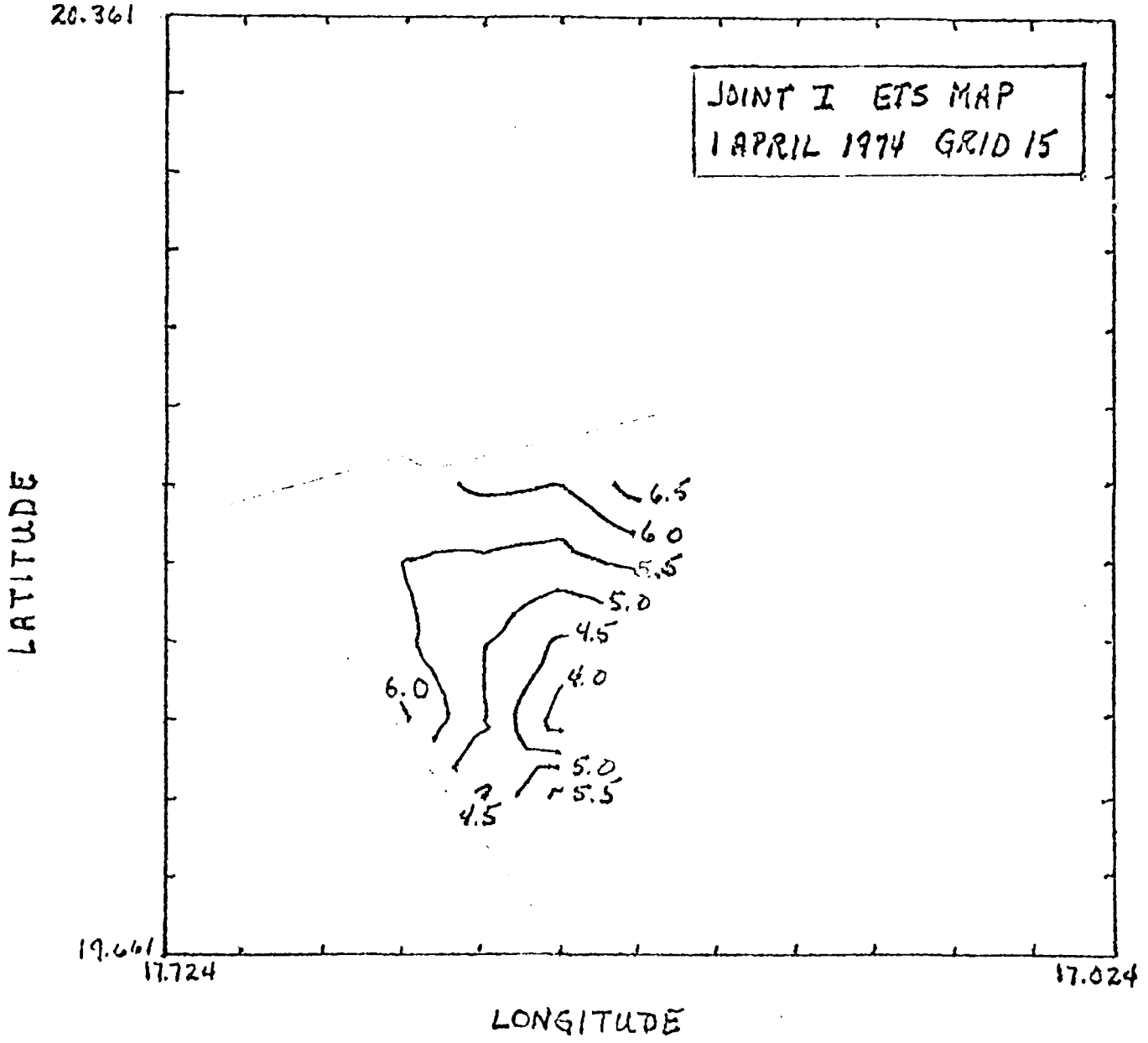

20.361
JOINT I ETS MAP
1 APRIL 1974 GRID 15
6.5
6.0
5.5
5.0
4.5
4.0
6.0
5.0
5.5
4.5
LATITUDE
19.661
17.724
17.024
LONGITUDE

Fig. 8.

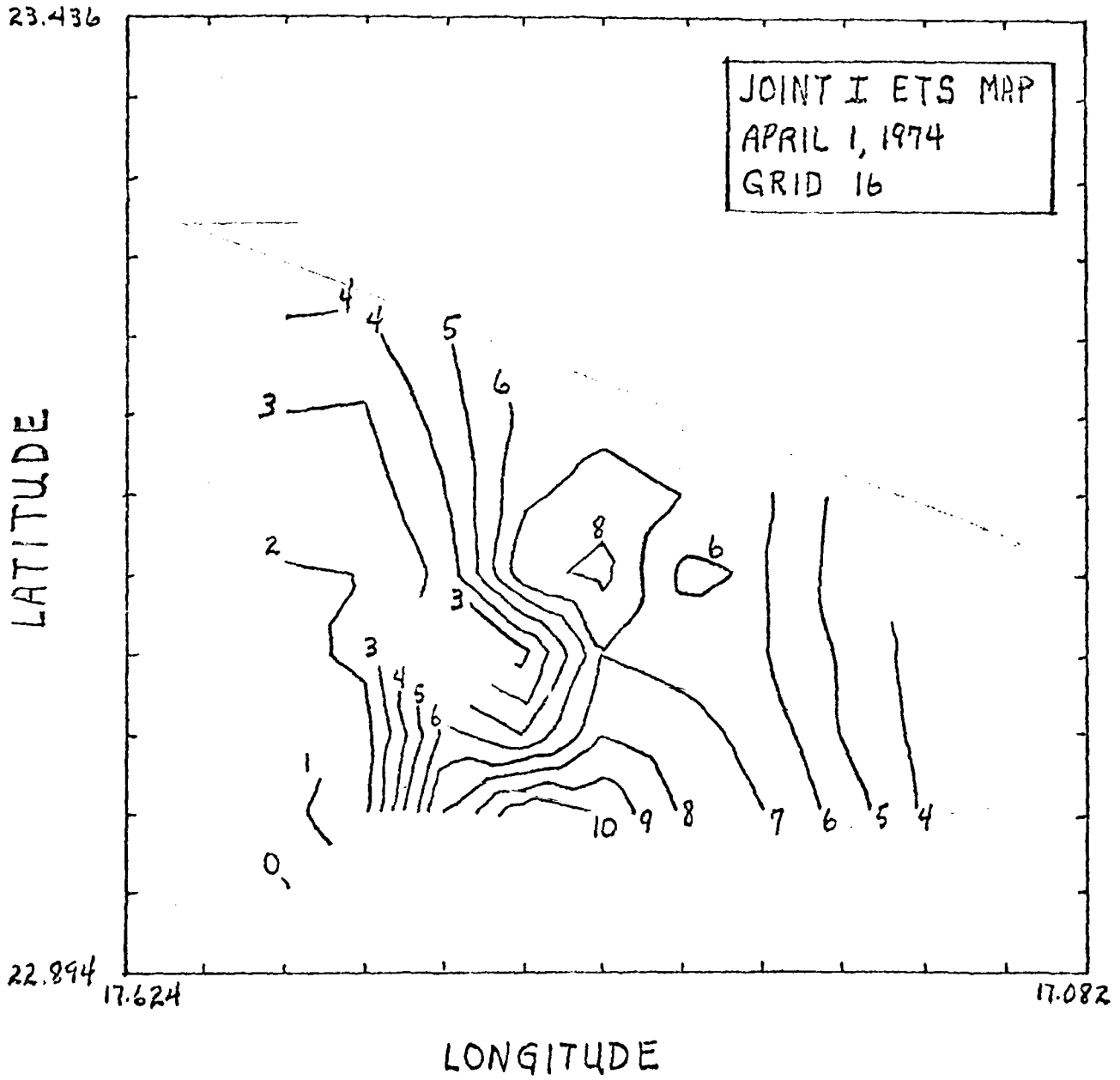

23.436
JOINT I ETS MAP
APRIL 1, 1974
GRID 16
LATITUDE
22.894
17.624
17.082
LONGITUDE

Fig. 9.

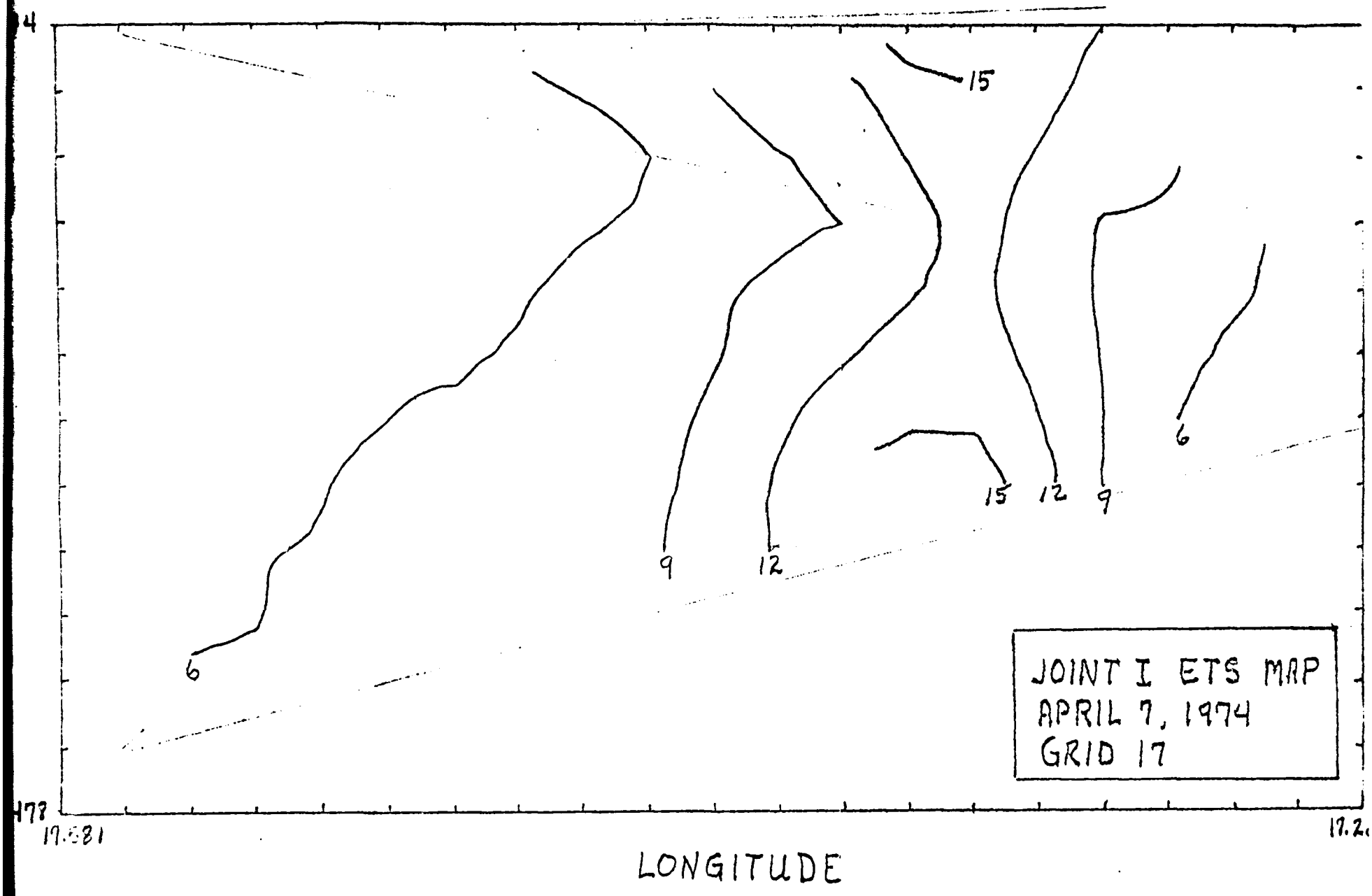

4
15
6
9
12
15
12
9
6
JOINT I ETS MAP
APRIL 7, 1974
GRID 17
H78
19.581
17.2
LONGITUDE

Fig. 10.

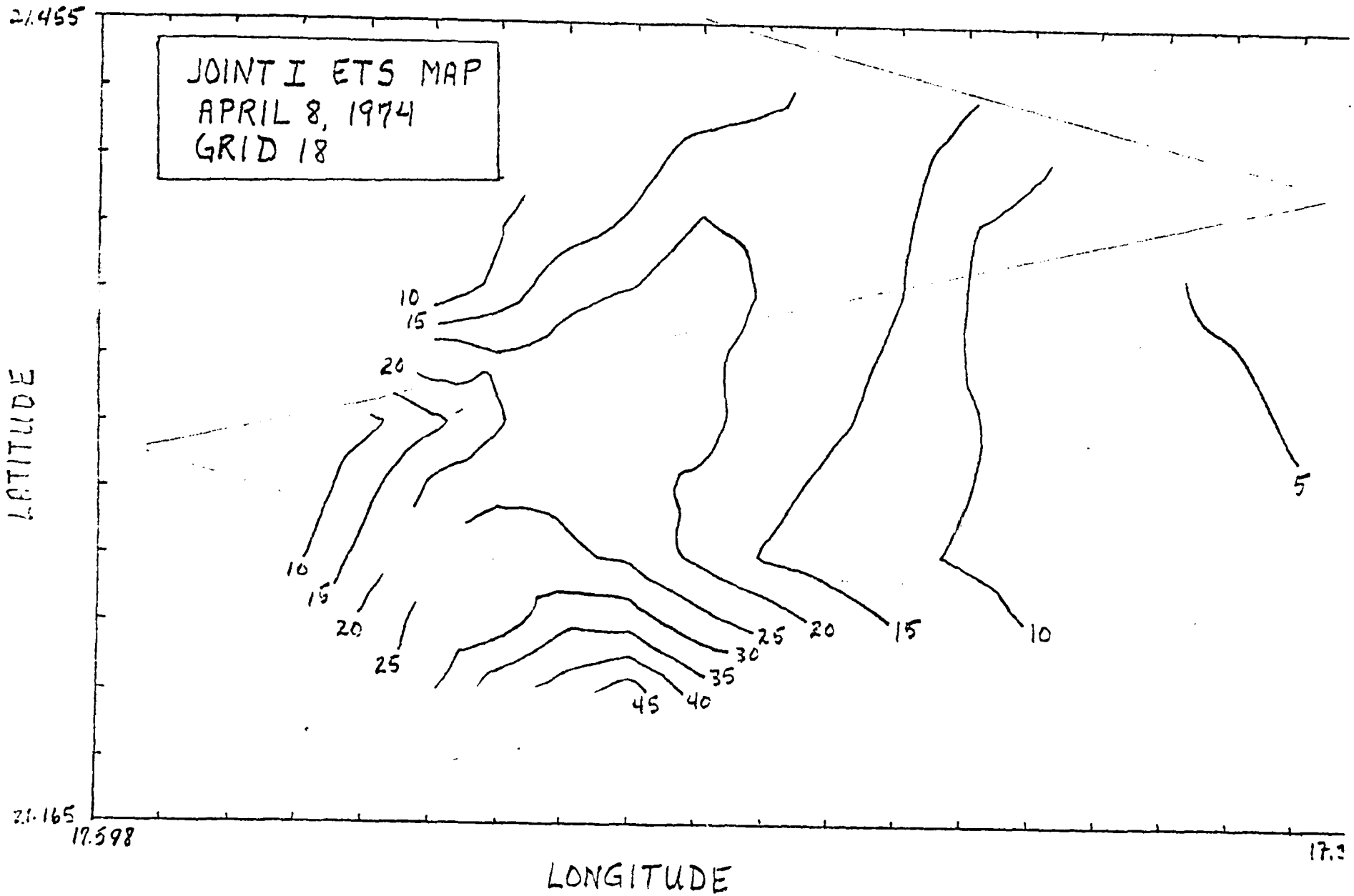

JOINT I ETS MAP
APRIL 8, 1974
GRID 18
21.455
21.165
17.598
LATITUDE
LONGITUDE
10
15
20
25
30
35
40
45
5

Fig. 11.

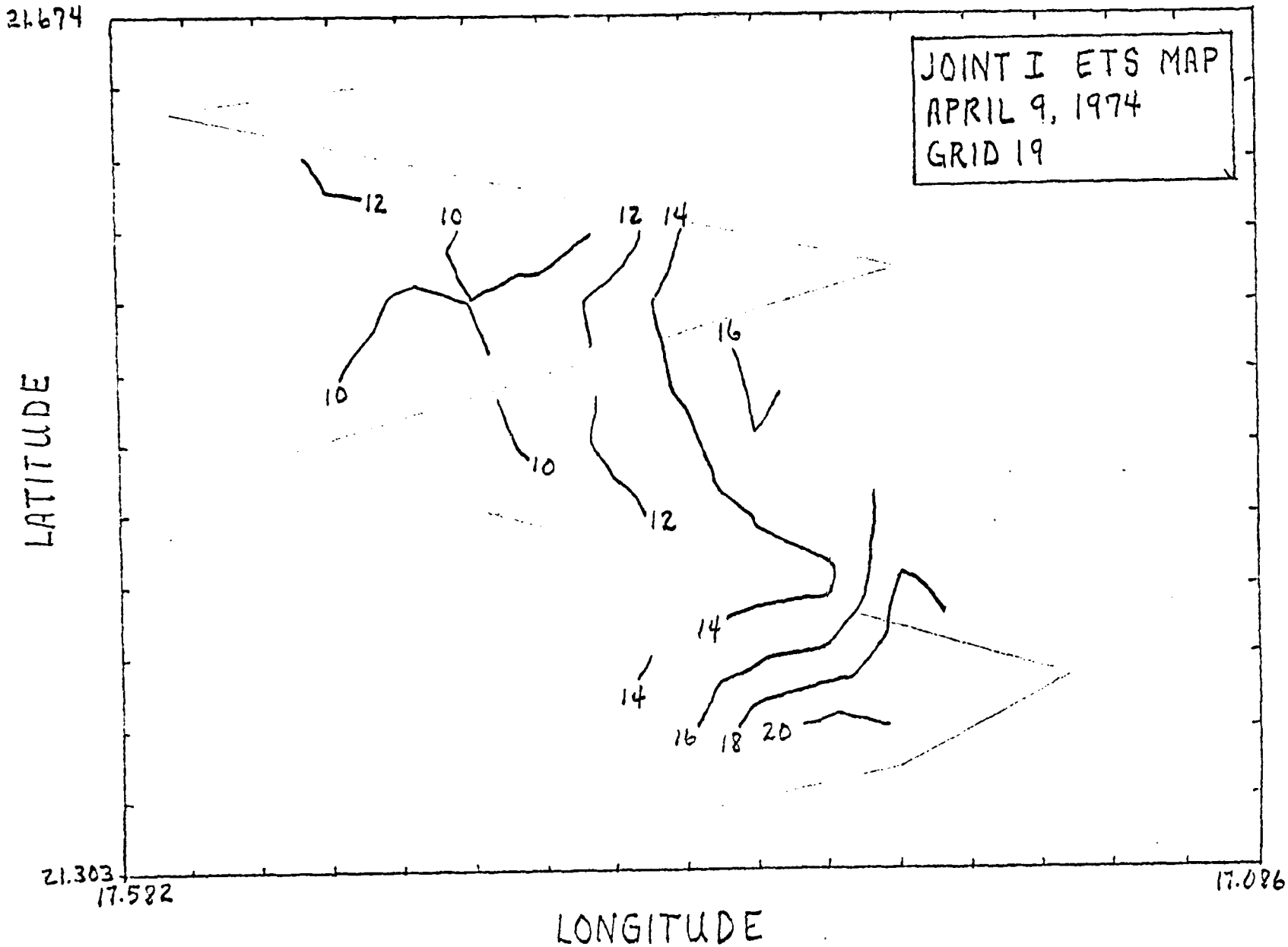

JOINT I ETS MAP
APRIL 9, 1974
GRID 19
21.674
21.303
17.582
17.086
LATITUDE
LONGITUDE
12
10
12
14
16
10
10
12
14
14
16
18
20

Fig. 12.

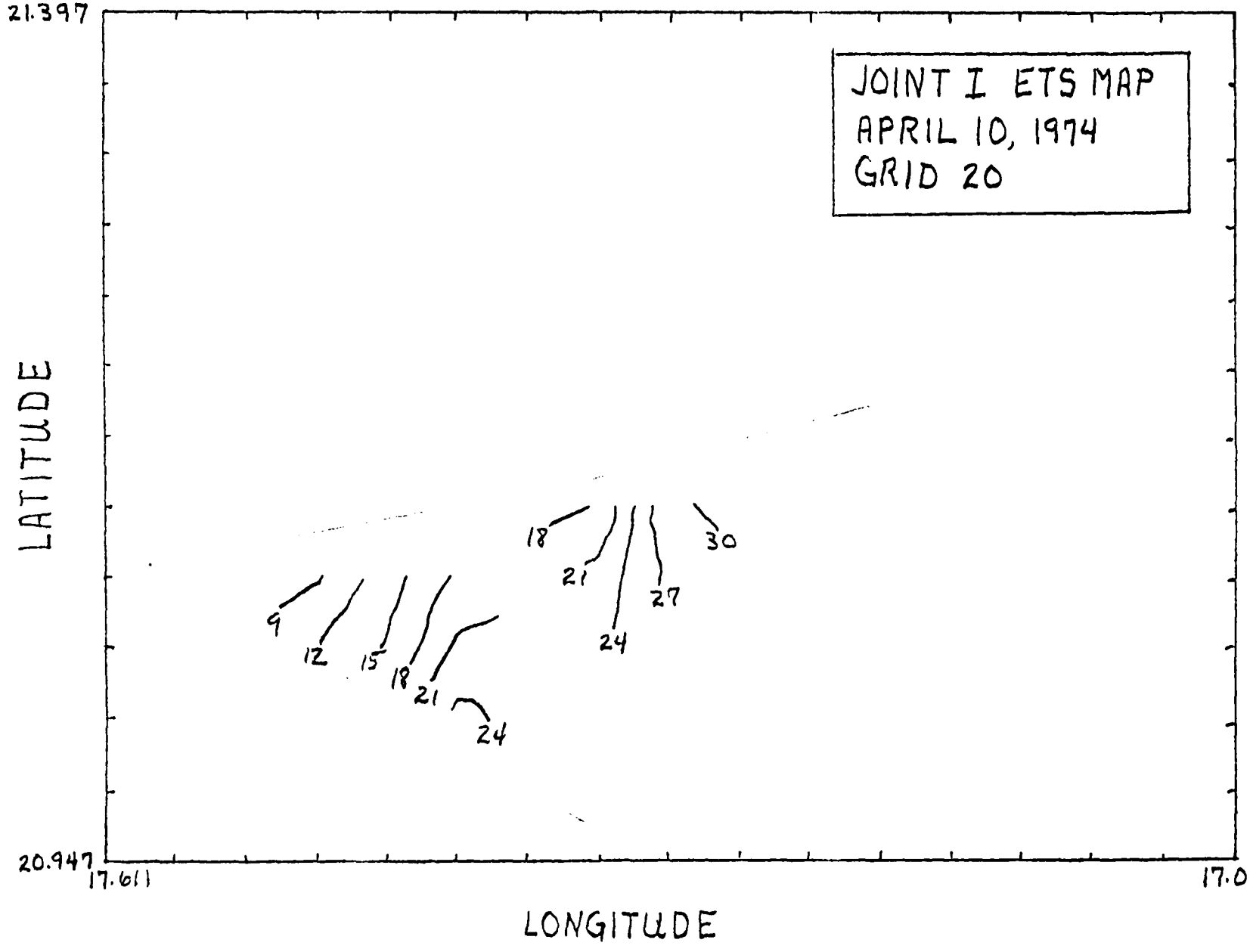

JOINT I ETS MAP
APRIL 10, 1974
GRID 20
21.397
20.947
17.611
17.011
LATITUDE
LONGITUDE
9
12
15
18
21
24
18
21
24
27
30

Fig. 13.

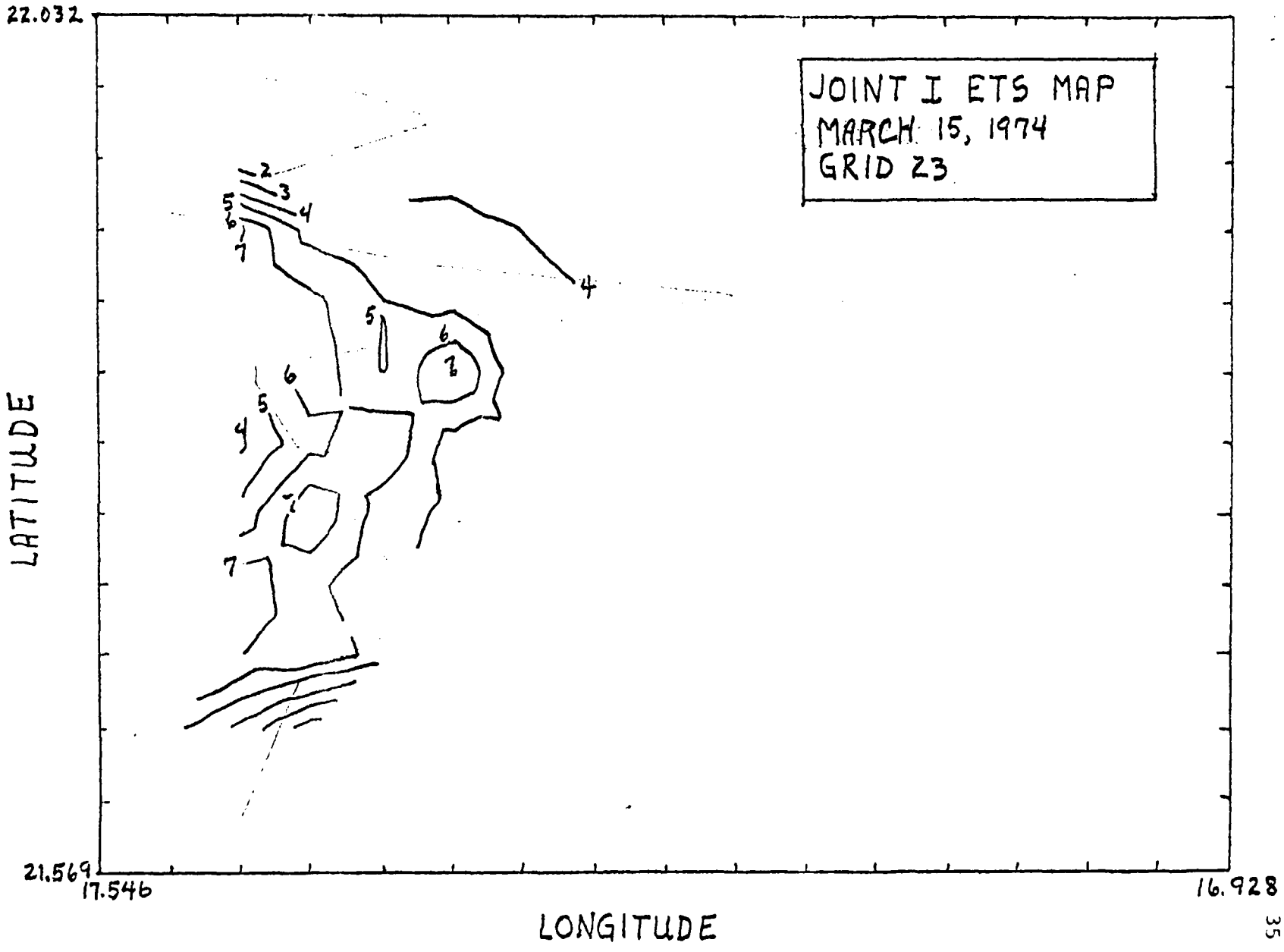

22.032
JOINT I ETS MAP
MARCH 15, 1974
GRID 23
LATITUDE
21.569
17.546
16.928
LONGITUDE

Fig. 14.

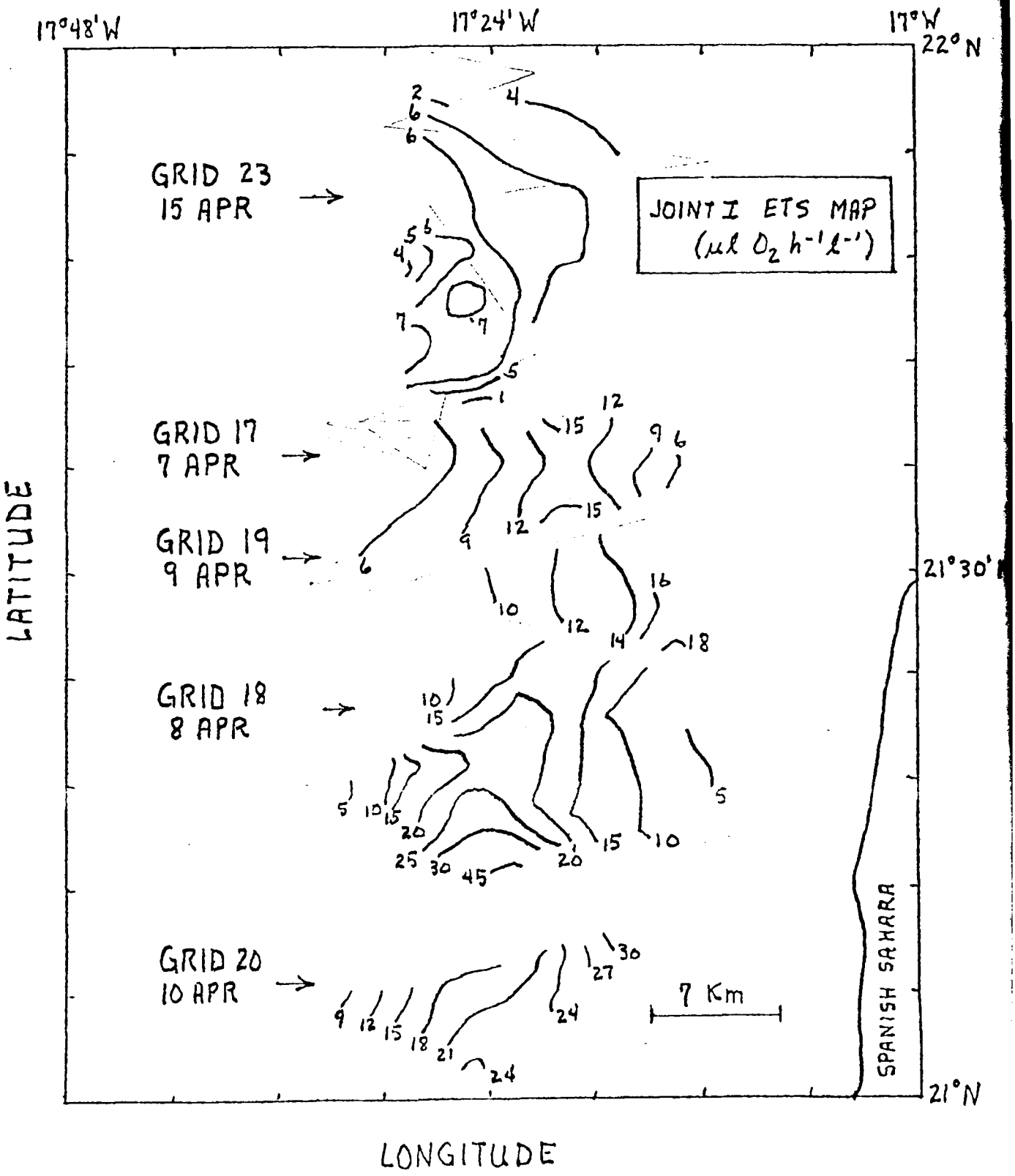

17°48'W
17°24'W
17°W
22°N
21°30'
21°N
LATITUDE
LONGITUDE
GRID 23
15 APR
GRID 17
7 APR
GRID 19
9 APR
GRID 18
8 APR
GRID 20
10 APR
JOINT I ETS MAP
(μl O2 h-1 l-1)
7 Km
SPANISH SAHARA

Fig. 15.

Table 1. Positions of the leg end-points for the mapping surveys on Leg II of the R/V Atlantis II phase of JOINT I.

GRID	POINT	LONGITUDE	LATITUDE	COMMENT
15	1	17.364	20.059	
	2	17.672	19.991	
	3	17.450	19.701	
	4	17.339	19.976	
16	1	17.593	23.316	
	2	17.114	23.139	
	3	17.555	23.10	
	4	17.127	22.987	
	5	17.557	22.929	
17	1	17.232	21.648	
	2	17.561	21.642	
	3	17.142	21.578	
	4	17.565	21.491	
18	1	17.400	21.460	
	2	17.168	21.40	
	3	17.585	21.30	
	4	17.175	21.252	
	5	17.500	21.182	
19	1	17.562	21.633	
	2	17.245	21.565	
	3	17.510	21.483	
	4	17.169	21.382	
	5	17.328	21.329	
	L1	17.040	21.315	
	L2	16.994	21.519	
	L3	16.966	21.612	
20	L1	17.074	20.873	Cape Blanc
	1	17.208	21.193	
	2	17.587	21.107	
	3	17.387	20.965	
23	1	17.468	21.600	
	2	17.526	21.640	
	3	17.364	21.716	
	4	17.460	21.852	
	5	17.196	21.887	
	6	17.507	21.928	
	7	17.356	21.971	
	8	17.468	21.996	
	L1	16.953	21.769	Cabo Corbiero

Table 2. Types of data collected during leg II of the R/V ATLANTIS II phase of the JOINT I expedition.

Grid Number	Temp.	CHL	NO_2^-	NO_3^-	SiO_4^-	PO_4^{3}	NH_4^+	Time	Acoustic A/B	Underway ETS
1	-	-	-	-	-	-	-		-	X
2	X	X	-	X	X	X	-	X		X
3	X	-	X	X	X	X	X	-		X
4	X	X	X	X	X	X	X	X		X
5	X	X	X	X	X	X	X	X		X
6	X	X	X	-	X	X	X	X		X
7	X	X	X	X	X	X	X	X		X
8	X	X	X	X	X	X	X	-		-
9	----------	----------	----------	----------	----------	no data	----------	----------	----------	X
10	X	X	X	X	X	X	X	-	-	

microheterotrophs (bacteria, protozoans, metazoan larvae) were present at these areas and were largely responsible for the relatively high levels of ETS activity. At grids 17 and 18 the correlation coefficient was very high, indicating relatively small abundance of microheterotrophs as compared to phytoplankton. These plots are combined and compared to the plot of the correlation coefficient of the regression of C-14 or chl-a in Fig. 17. A similar pattern parallels all three profiles suggesting that the microheterotrophs explain only part of the coupling or uncoupling between ETS activity and the phytoplankton indices.

Summaries of the analysis of variance on all three regressions (Tables 3, 4, and 5) are given in Tables 6, 7, and 8.

Table 3. Statistics for the regression of C-14 uptake on ETS activity. Abbreviations: b=intercept; m=regression coefficient; SER=standard error of the regression coefficient; T=T-Value; r=correlation coefficient; SEE=standard error of estimate.

STATISTIC	GRID NUMBER						
	15	16	17	18	19	20	23
b	1.88	20.02	4.40	5.54	4.28	9.56	0.21
m	0.142	0.44	1.56	1.12	1.02	0.48	0.012
SER	0.068	0.13	0.087	0.048	0.073	0.034	0.0037
T	2.10	3.42	17.99	23.41	13.95	14.10	3.16
r	0.17	0.21	0.81	0.87	0.67	0.64	0.22
SEE	0.69	6.22	5.13	4.77	5.60	5.29	0.076

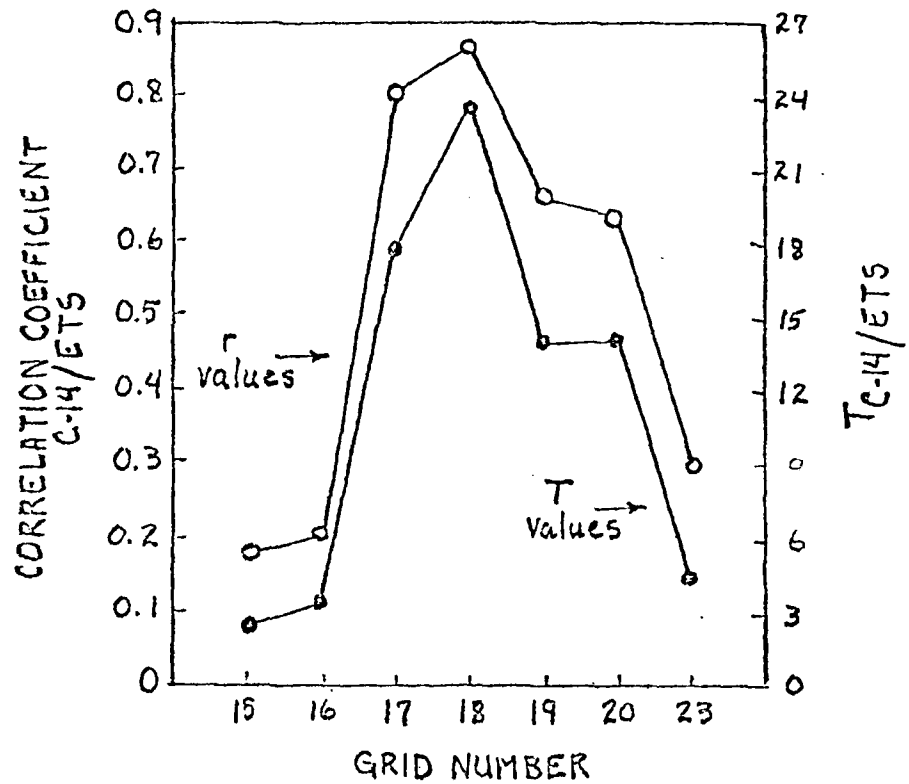
Table 4. Statistics for the regression of Chl-a on ETS activity. Abbreviations: b=intercept; m=regression coefficient; SER=standard error of the regression coefficient; T=T-Value; r=correlation coefficient; SEE=standard error of estimate.

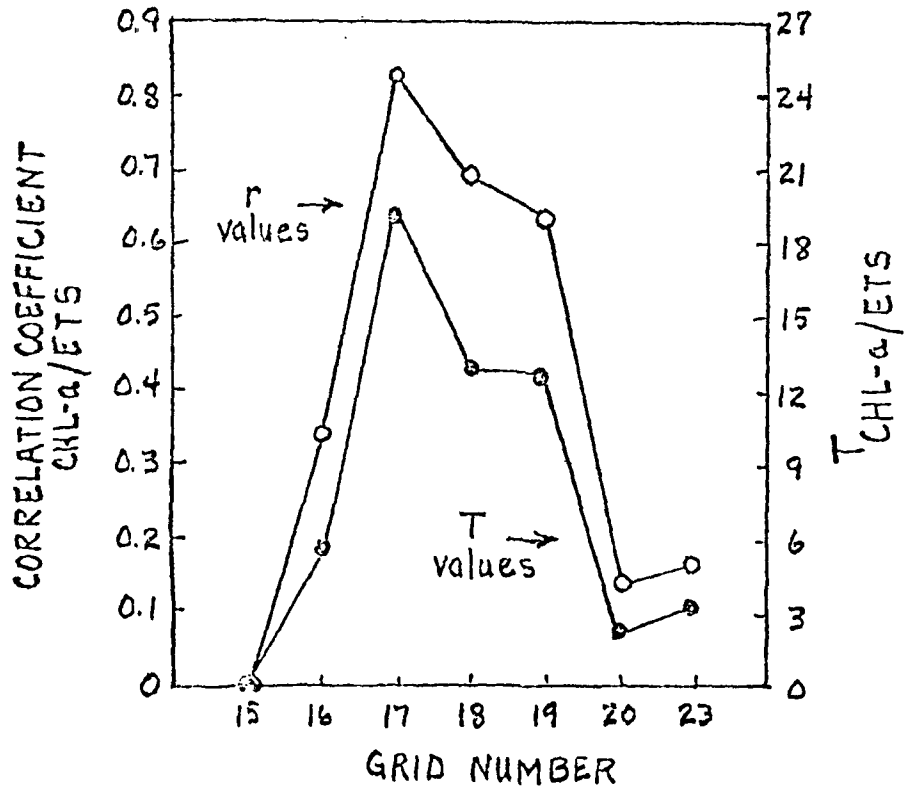
STATISTIC	GRID NUMBER						
	15	16	17	18	19	20	23
b	0.16	0.18	0.13	0.12	0.14	0.31	3.32
m	0.0015	0.0061	0.015	0.013	0.014	0.00096	0.53
SER	0.0082	0.0010	0.00076	0.0010	0.0011	0.00042	0.12
T	0.19	5.85	19.39	12.85	12.87	2.28	4.59
r	0.016	0.34	0.83	0.69	0.64	0.13	0.31
SEE	0.084	0.051	0.044	0.10	0.081	0.065	2.41

Table 5. Statistics for the regression of C-14 uptake on Chl-a. Abbreviations: b=intercept; m=regression coefficient; SER=standard error of the regression coefficient; T=T-Value; r=correlation coefficient; SEE=standard error of estimate.

STATISTIC	GRID NUMBER						
	15	16	17	18	19	20	23
b	2.70	16.39	-4.64	3.34	-1.65	1.66	3.44
m	0.12	28.12	86.30	55.73	61.23	47.30	9.78
SER	0.69	7.08	5.19	2.98	2.40	5.59	2.20
T	0.18	3.97	16.62	18.70	25.47	8.46	4.45
r	0.015	0.24	0.79	0.81	0.85	0.45	0.30
SEE	0.70	6.17	5.38	5.60	3.93	6.17	2.42

Fig. 16. Correlation coefficients and T-values for the ^{14}C-ETS and Chl-a-ETS regressions.

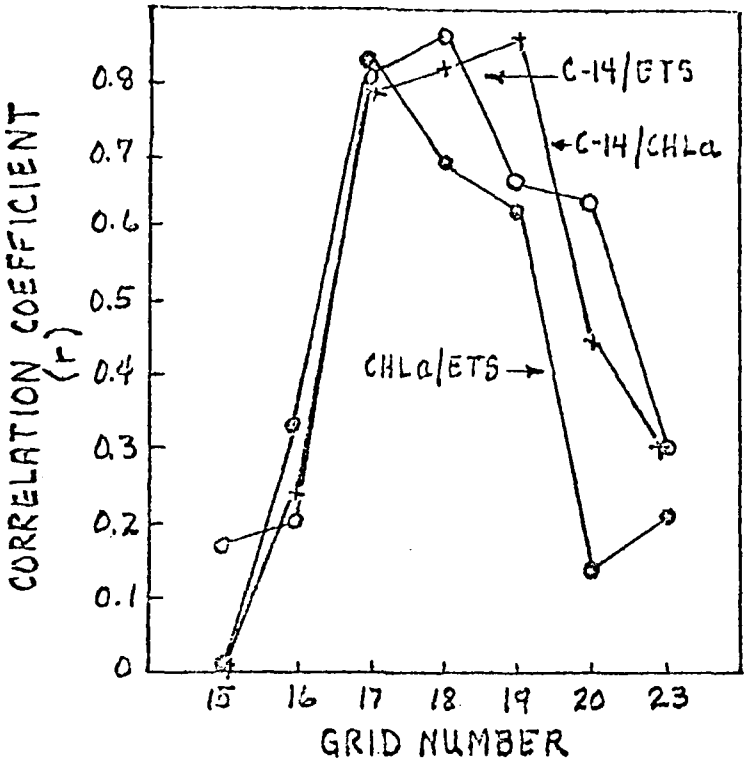




JOINT-I LEG II

R.V. ATLANTIS II

Fig. 17. Correlation coefficients from the 3 regressions shown in Tables 3, 4, and 5 (top). The slope and the intercept for the regression of ^{14}C-uptake on Chl-a (bottom).


CORRELATION COEFFICIENT (r)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
C-14/ETS
C-14/CHLa
CHLa/ETS
15
16
17
18
19
20
23
GRID NUMBER

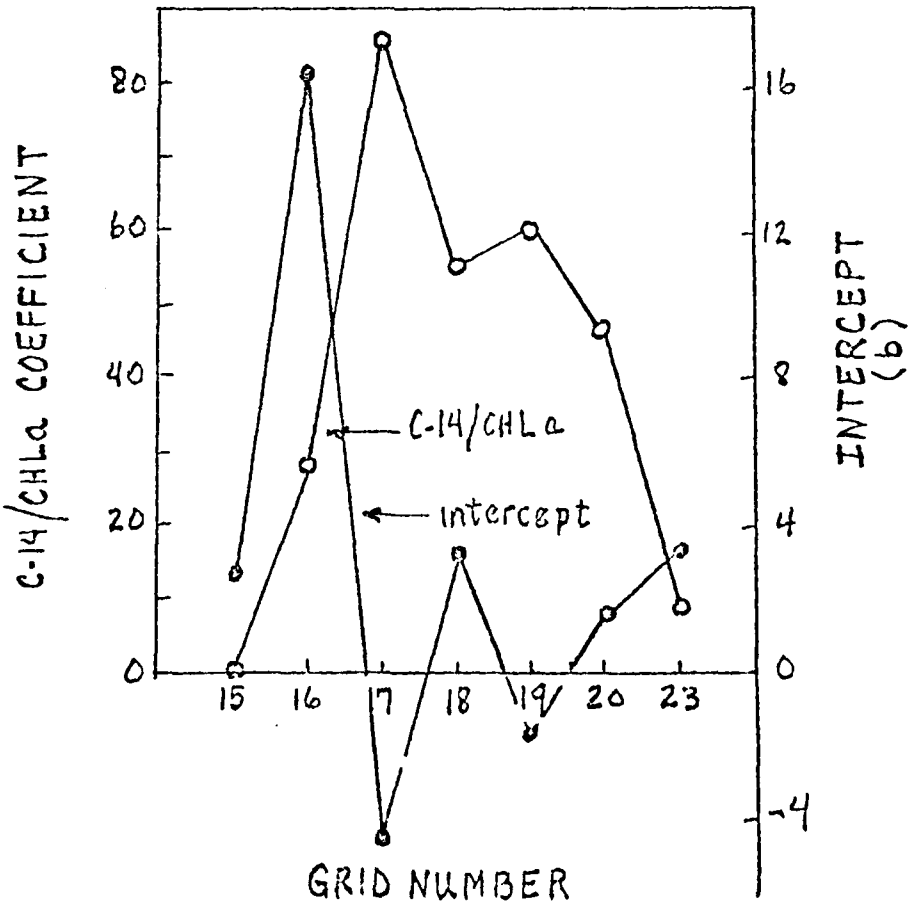
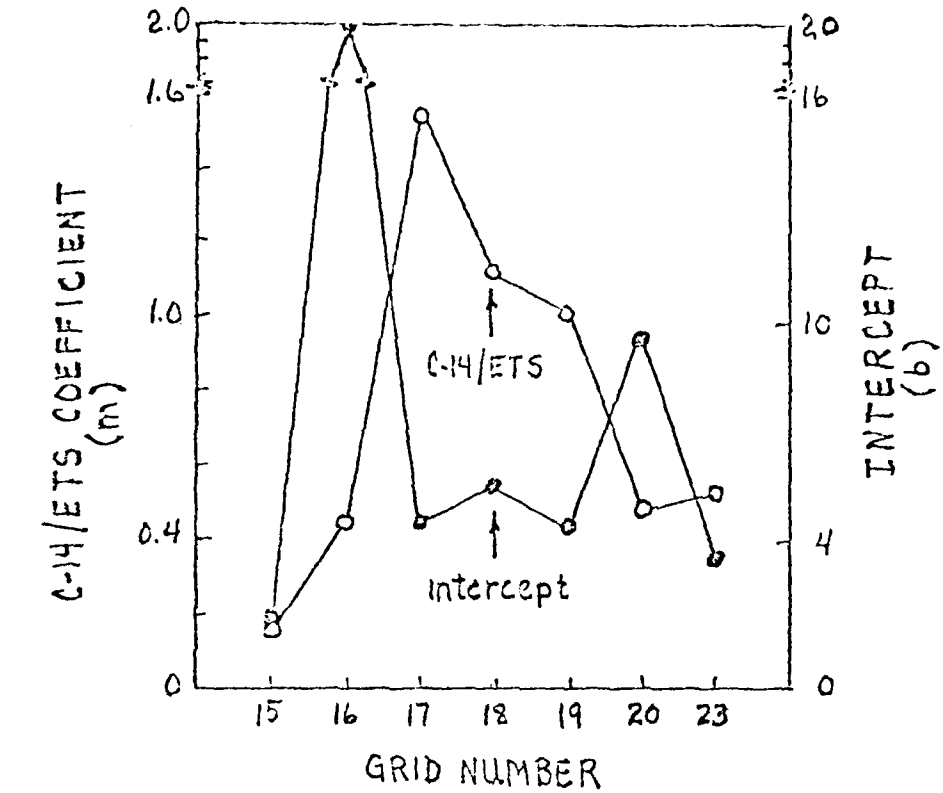
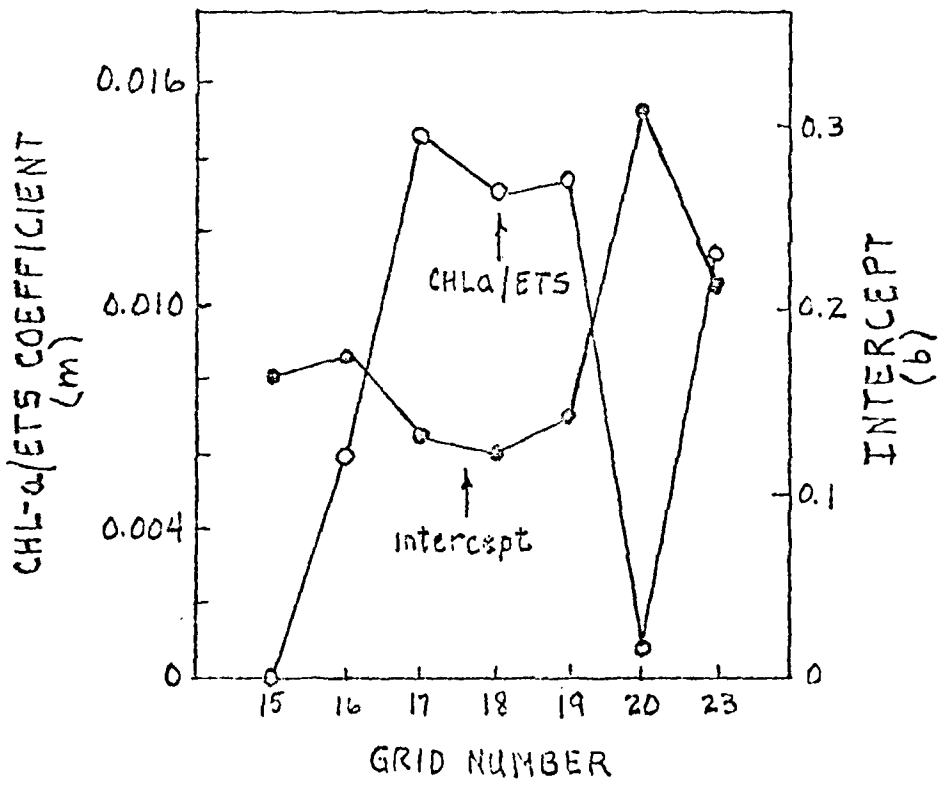

C-14/CHLa COEFFICIENT
80
60
40
20
0
C-14/CHLa
intercept
INTERCEPT (b)
16
12
8
4
0
-4
15
16
17
18
19
20
23
GRID NUMBER

Fig. 18. As in the bottom panel of Fig. 17.





JOINT-I LEG II

R.V. ATLANTIS II

Table 6. Analysis of variance for the regression of C-14 uptake on ETS activity. Abbreviations: VAR= variance attributable to the regression; VDR= variance deviation from the regression; DR= degrees of freedom; and SS, MS, and F- value have their usual meanings.

	DF	SS	MS	F-VALUE
GRID 15:				
VAR	1	2.13	2.13	4.43
VDR	145	69.91	0.48	
TOTAL	146	72.04		
GRID 16:				
VAR	1	452.80	452.80	11.71
DR	257	9938.7	38.67	
TOTAL	258	10392.		
GRID 17:				
VAR	1	8422.6	8422.6	320.31
VDR	168	4417.6	26.30	
TOTAL	169	12840.		
GRID 18:				
VAR	1	12492.	12492.	548.08
VDR	179	4079.8	22.79	
TOTAL	180	16572.		
GRID 19:				
VAR	1	6106.6	6106.6	194.47
VDR	245	7693.4	31.40	
TOTAL	246	13800.		
GRID 20:				
VAR	1	5553.5	5553.5	198.74
VDR	280	7824.1	27.94	
TOTAL	281	13378.		
GRID 23:				
VAR	1	0.057	0.057	9.97
VDR	200	1.15	0.0057	
TOTAL	201	1.21		

Table 7. Analysis of variance for the regression of Chl-a on ETS activity. Abbreviations: VAR= variance attributable to the regression; VDR=variance deviation from the regression; DF=degrees of freedom; and SS, MS, and F-value have their usual meanings.

	DF	SS	MS	F-VALUE
GRID 15:				
VAR				0.031
VDR		NO DATA		
TOTAL				
GRID 16:				
VAR	1	0.089	0.089	34.23
VDR	257	0.67	0.0026	
TOTAL	258	0.76		
GRID 17:				
VAR	1	0.74	0.74	375.99
VDR	168	0.33	0.0020	
TOTAL	169	1.07		
GRID 18:				
VAR	1	1.69	1.69	165.19
VDR	179	1.84	0.010	
TOTAL	180	3.53		
GRID 19:				
VAR	1	1.08	1.08	165.59
VDR	245	1.59	0.0065	
TOTAL	246	2.67		
GRID 20:				
VAR	1	0.022	0.022	5.19
VDR	280	1.20	0.0043	
TOTAL	281	1.22		
GRID 23:				
VAR	1	122.20	122.20	21.05
VDR	200	1161.0	5.81	
TOTAL	201	1283.2		

Table 8. Analysis of variance for the regression of C-14 uptake on Chl-a. Abbreviations: VAR=variance attributable to the regression; VDR=variance deviation from the regression; DF=degrees of freedom; and SS, MS, and F-value have their usual meanings.

	DF	SS	MS	F-VALUE
GRID 15:				
VAR	1	0.015	0.015	0.031
VDR	145	72.02	0.50	
TOTAL	146	72.04		
GRID 16:				
VAR	1	601.35	601.35	15.79
VDR	257	9790.2	38.09	
TOTAL	258	10392.		
GRID 17:				
VAR	1	7984.9	7984.9	276.29
VDR	168	4855.2	28.90	
TOTAL	169	12840.		
GRID 18:				
VAR	1	10961.	10961.	349.65
VDR	179	5611.2	31.35	
TOTAL	180	16572.		
GRID 19:				
VAR	1	10016.	10016.	648.57
VDR	245	3783.7	15.44	
TOTAL	246	13800.		
GRID 20:				
VAR	1	2725.2	2725.2	71.63
VDR	280	10652.	38.05	
TOTAL	281	13378.		
GRID 23:				
VAR	1	115.38	115.38	19.76
VDR	200	1167.8	5.84	
TOTAL	201	1283.2		

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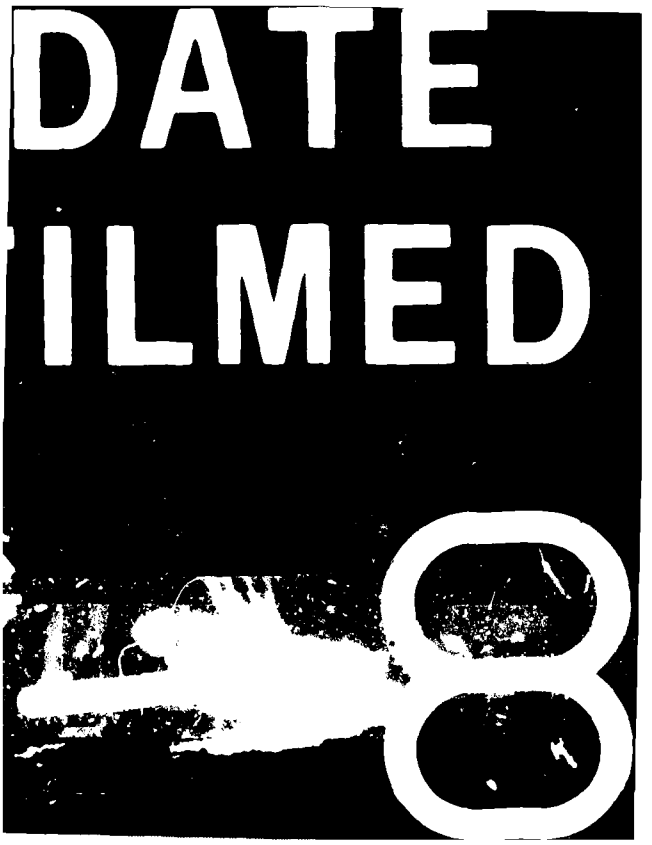
ACKNOWLEDGEMENTS

This research has been supported by NSF grant numbers OCE 75-23718 and OCE 7300610 (COSA-12) and ONR contract number N00014-76-C-0271 to T. T. Packard.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